

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Hidenori OHKI, et al.

GAU:

SERIAL NO: NEW APPLICATION

EXAMINER:

FILED: HERewith

FOR: CEPHEM COMPOUNDS

REQUEST FOR PRIORITY

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

- ☐ Full benefit of the filing date of U.S. Application Serial Number , filed , is claimed pursuant to the provisions of 35 U.S.C. §120.
- ☐ Full benefit of the filing date(s) of U.S. Provisional Application(s) is claimed pursuant to the provisions of 35 U.S.C. §119(e):
Application No. Date Filed
- ☒ Applicants claim any right to priority from any earlier filed applications to which they may be entitled pursuant to the provisions of 35 U.S.C. §119, as noted below.

In the matter of the above-identified application for patent, notice is hereby given that the applicants claim as priority:


<u>COUNTRY</u>	<u>APPLICATION NUMBER</u>	<u>MONTH/DAY/YEAR</u>
Australia	2002952355	October 30, 2002
Australia	2003904813	September 4, 2003

Certified copies of the corresponding Convention Application(s)

- ☒ are submitted herewith
- ☐ will be submitted prior to payment of the Final Fee
- ☐ were filed in prior application Serial No. filed
- ☐ were submitted to the International Bureau in PCT Application Number
Receipt of the certified copies by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.
- ☐ (A) Application Serial No.(s) were filed in prior application Serial No. filed ; and
- ☐ (B) Application Serial No.(s)
☐ are submitted herewith
☐ will be submitted prior to payment of the Final Fee

Respectfully Submitted,

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Patent Office
Canberra

I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002952355 for a patent by FUJISAWA PHARMACEUTICAL CO., LTD. and WAKUNAGA PHARMACEUTICAL CO., LTD. as filed on 30 October 2002.

WITNESS my hand this
Eighth day of October 2003

A handwritten signature in cursive script that reads "J R Yabsley".

JONNE YABSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Fujisawa Pharmaceutical Co., Ltd.
and
Wakunaga Pharmaceutical Co., Ltd.

A U S T R A L I A
Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"Cephem Compounds"

The invention is described in the following statement:

DESCRIPTION
CEPHEM COMPOUNDS
TECHNICAL FIELD

The present invention relates to new cephem
5 compounds and pharmaceutically acceptable salts thereof.
More particularly, the present invention relates to new
cephem compounds and pharmaceutically acceptable salts
thereof, which have antimicrobial activities; to
processes for preparation thereof, to pharmaceutical
10 composition comprising the same, and to a method for
treating infectious diseases in human being and animals.

DISCLOSURE OF INVENTION

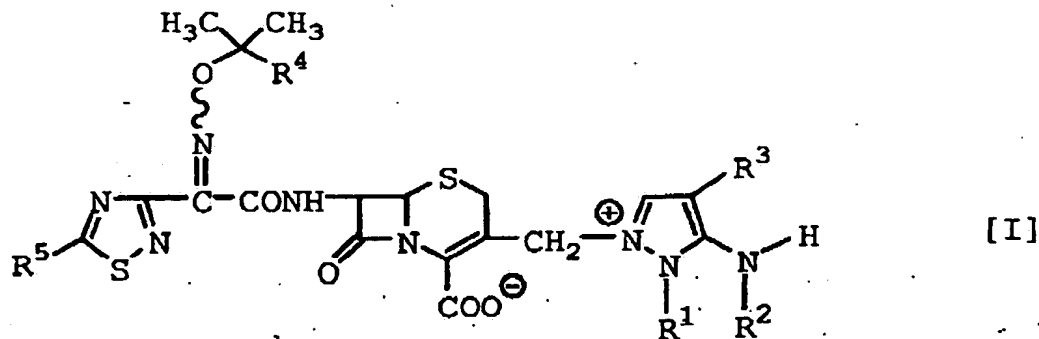
One object of the present invention is to provide
novel cephem compounds and pharmaceutically acceptable
15 salts thereof, which are highly active against a number
of pathogenic microorganisms.

Another object of the present invention is to
provide processes for the preparation of said cephem
compounds and salts thereof.

20 A further object of the present invention is to
provide a pharmaceutical composition comprising, as an
active ingredient, said cephem compounds or their
pharmaceutically acceptable salts.

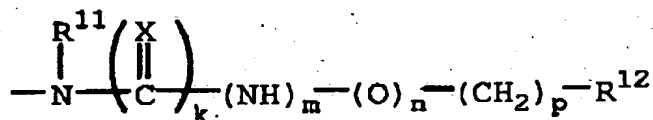
Still further object of the present invention is
25 to provide a method for treating infectious diseases
caused by pathogenic microorganisms, which comprises
administering said cephem compounds to infected human
being or animals.

The object cephem compounds of the present
30 invention are novel and can be represented by the
following general formula [I]:



wherein

- R^1 is lower alkyl or hydroxy(lower)alkyl, and
 5 R^2 is hydrogen or amino protecting group, or
 R^1 and R^2 are bonded together and form lower alkylene;
 R^3 is



wherein X is O or NH,

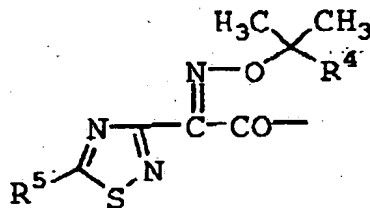
- 10 R^{11} is hydrogen or amino protecting group,
 R^{12} is amino or protected amino,
 k, m and n are independently 0 or 1, and
 p is 0, 1, 2 or 3;

R^4 is carboxy or protected carboxy; and

- 15 R^5 is amino or protected amino.

As to the object compound [I], the following points are to be noted.

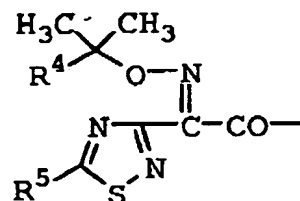
- That is, the object compound [I] includes syn isomer (Z form), anti isomer (E form) and a mixture thereof. Syn isomer (Z form) means one geometrical isomer having the partial structure represented by the following formula:



wherein R^4 and R^5 are each as defined above,

- 25 and anti isomer (E form) means the other geometrical

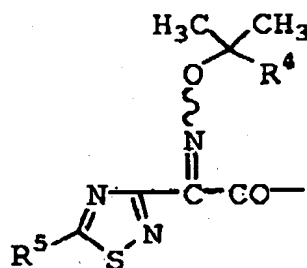
isomer having the partial structure represented by the following formula:



wherein R^4 and R^5 are each as defined above,

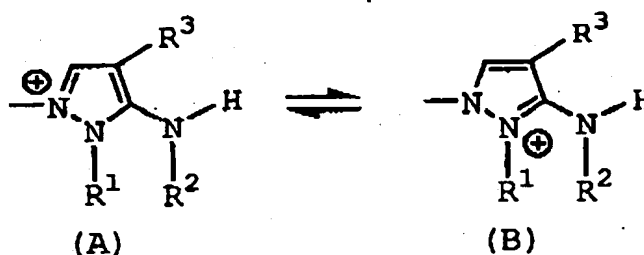
- 5 and all of such geometrical isomers and mixture thereof are included within the scope of this invention.

In the present specification and claims, the partial structure of these geometrical isomers and mixture thereof are represented for convenience' sake by the following formula:



wherein R^4 and R^5 are each as defined above.

- Another point to be noted is that the pyrazolio moiety of the compound [I] can also exist in the tautomeric form, and such tautomeric equilibrium can be represented by the following formula.

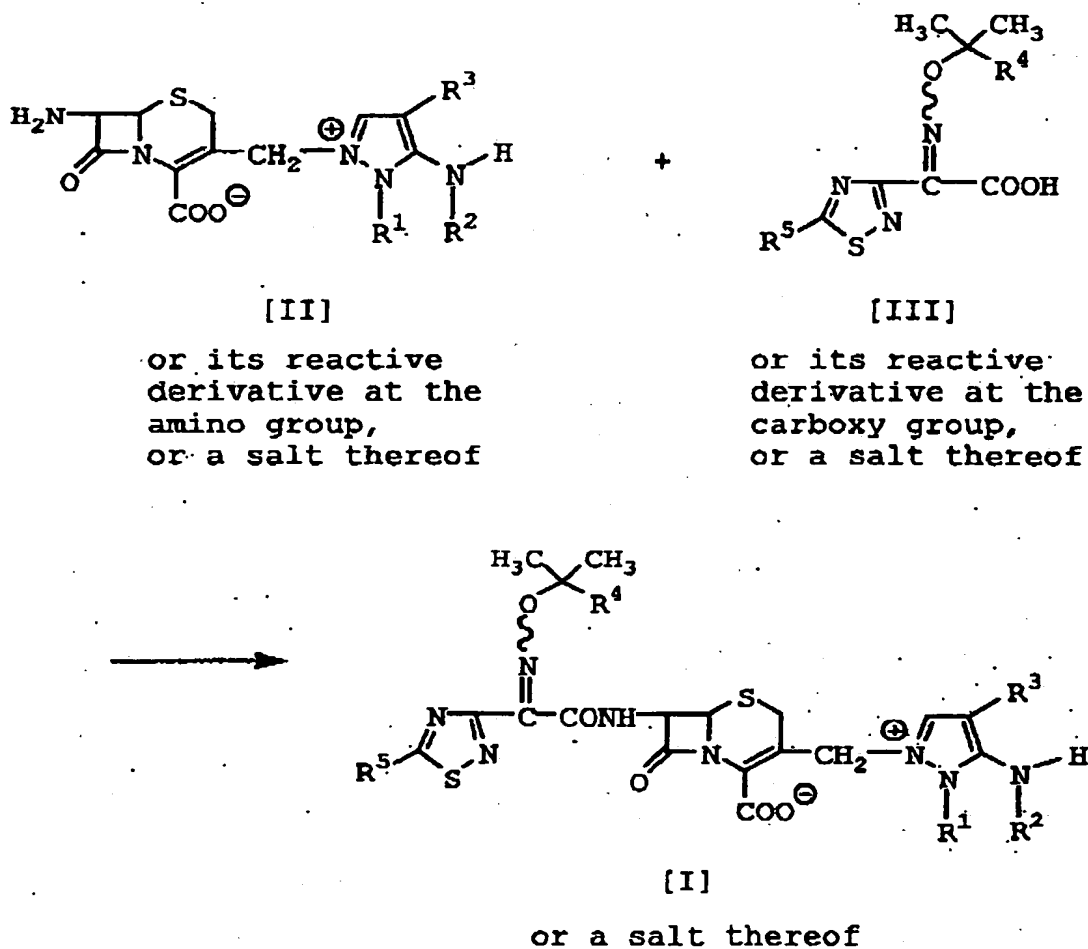


wherein R^1 , R^2 and R^3 are each as defined above.

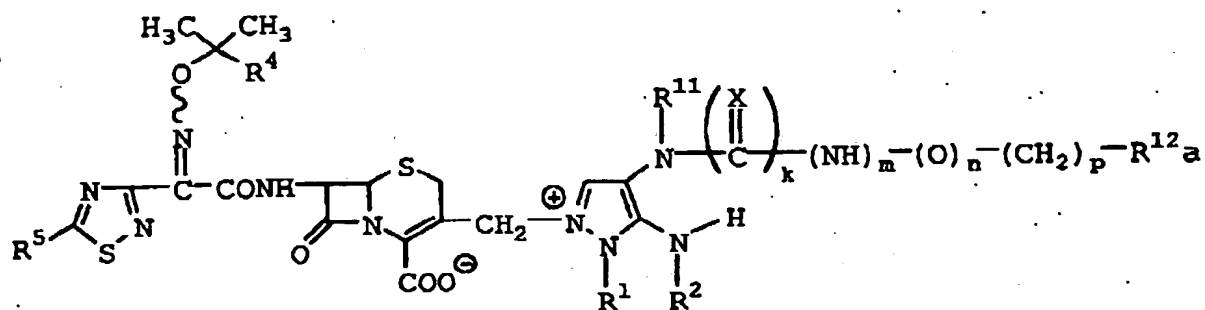
- Both of the above tautomeric isomers are included within the scope of the present invention, and in the present specification and claims, however, the object compound [I] is represented for convenience' sake by one expression of the pyrazolio group of the formula (A).

The cephem compound [I] of the present invention can be prepared by the following processes as illustrated in the following.

5 Process 1



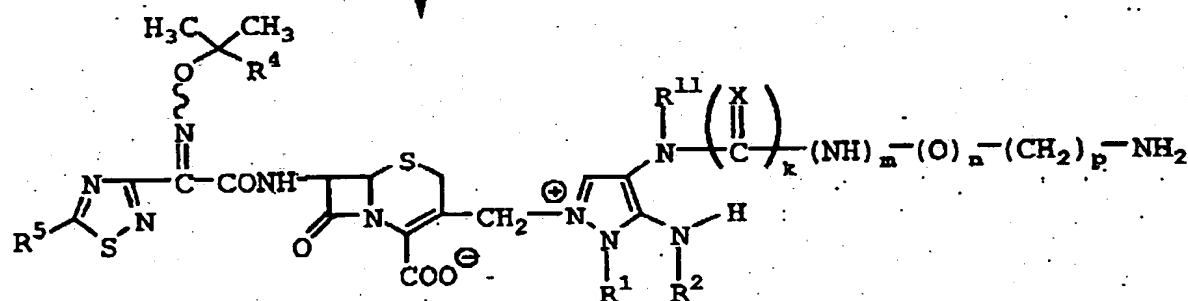
Process 2



[Ia]

or a salt thereof

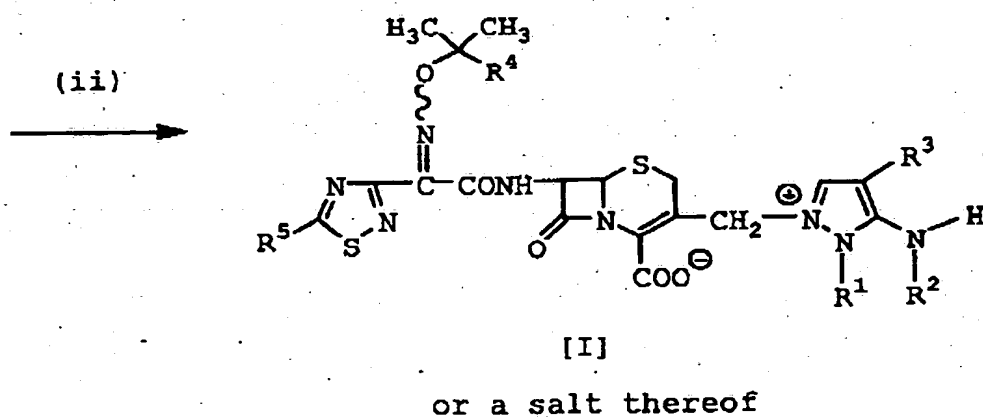
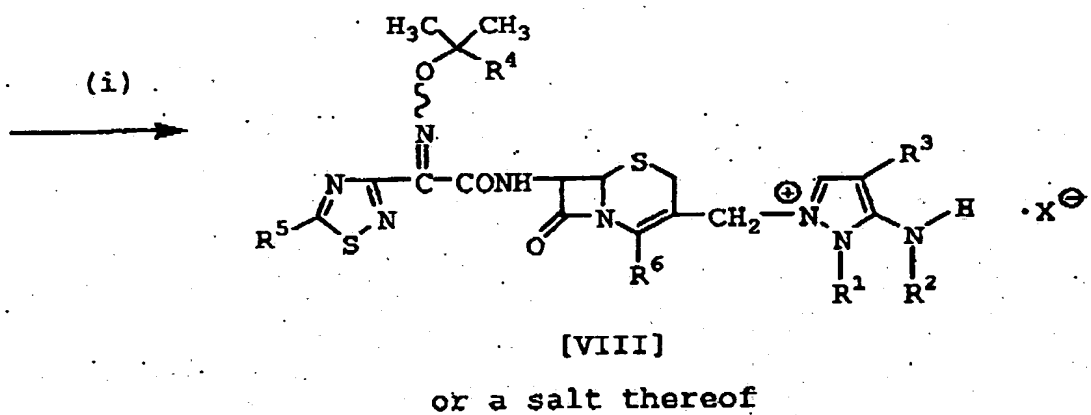
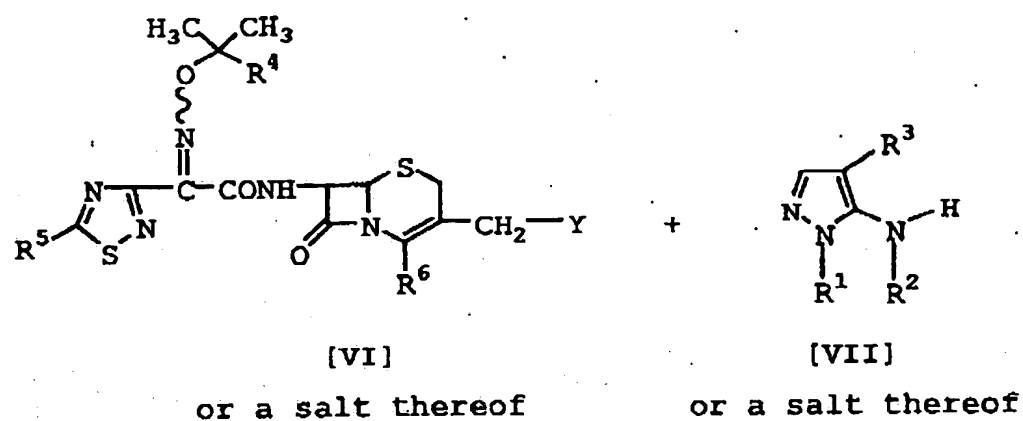
Elimination reaction of the
amino protecting group



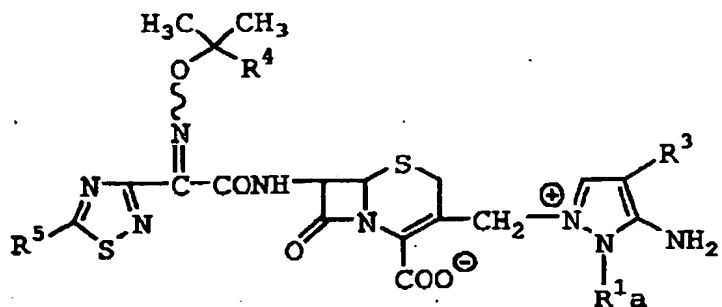
[Ib]

or a salt thereof

Process 3



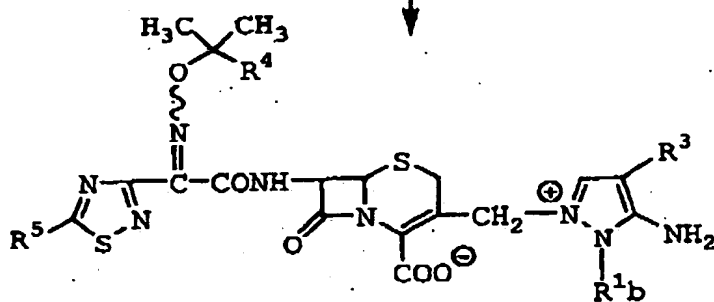
Process 4



[Ic]

or a salt thereof

Elimination reaction of
the hydroxy protecting
group



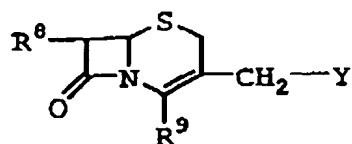
[Id]

or a salt thereof

- wherein R¹, R², R³, R⁴, R⁵, R¹¹, X, k, m, n, p are each as
 5 defined above,
 R⁶ is protected carboxy,
 Y is a leaving group,
 x[⊖] is an anion,
 R^{1a} is protected hydroxy(lower)alkyl,
 10 R^{1b} is hydroxy(lower)alkyl, and
 R^{12a} is protected amino.

The starting compounds [II] and [VI] can be prepared by the following processes.

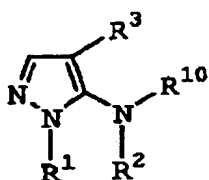
Process A



[X]

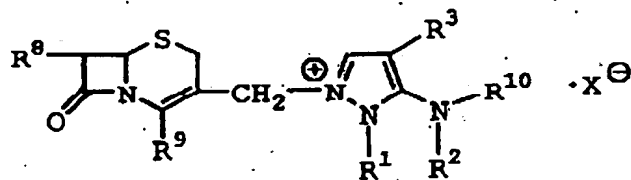
or a salt thereof

(i)



[XI]

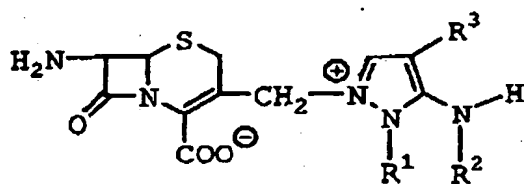
or a salt thereof



[XII]

or a salt thereof

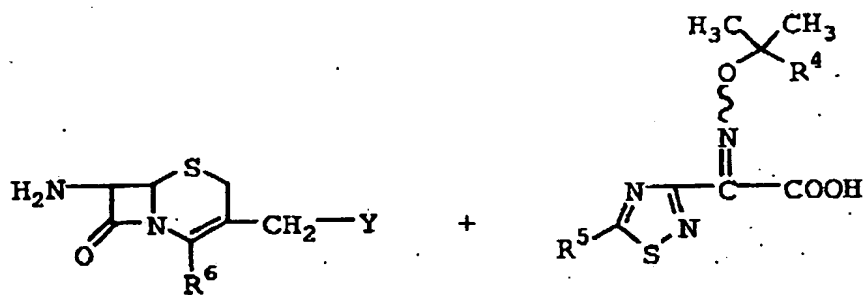
(ii)



[II]

or a salt thereof

Process B

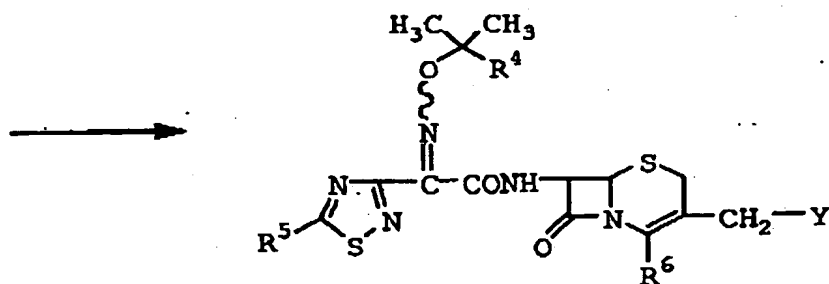


[XIII]

or its reactive
derivative at the
amino group,
or a salt thereof

[XIV]

or its reactive
derivative at the
carboxy group,
or a salt thereof



[VI]

or a salt thereof

wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , Y and X^\ominus are each as
5 defined above,

R^8 is protected amino,

R^9 is protected carboxy, and

R^{10} is amino protecting group.

The starting compounds [VII] and [XI] or salts
10 thereof can be prepared by the methods disclosed in the
Preparations 3-46 and 48-51 described later or similar
manners thereto.

In the above and subsequent descriptions of this
specification, suitable examples of the various
15 definitions are explained in detail as follows.

The term "lower" is used to mean a group having 1

to 6, preferably 1 to 4, carbon atom(s), unless otherwise indicated.

Suitable "lower alkyl" and "lower alkyl" moiety in "hydroxy(lower)alkyl" and "protected

5 hydroxy(lower)alkyl" include straight or branched alkyl having 1 to 6 carbon atom(s), such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, tert-pentyl and hexyl, in which more preferred one is C₁-C₄ alkyl.

10 Suitable "hydroxy(lower)alkyl" includes hydroxy(C₁-C₆)alkyl such as hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 4-hydroxybutyl, 5-hydroxypentyl and 6-hydroxyhexyl, in which more
15 preferred one is hydroxy(C₁-C₄)alkyl.

Suitable "lower alkylene" formed by R¹ and R² includes straight alkylene having 1 to 6, preferably 2 to 4 carbon atoms, such as methylene, ethylene, trimethylene and tetramethylene, in which more preferred
20 one is straight alkylene having 2 or 3 carbon atoms.

Suitable "amino protecting group" in "protected amino" includes an acyl group as mentioned below, substituted or unsubstituted aryl(lower)alkylidene [e.g., benzylidene, hydroxybenzylidene, etc.], aryl(lower)alkyl
25 such as mono-, di- or triphenyl(lower)alkyl [e.g., benzyl, phenethyl, benzhydryl, trityl, etc.], and the like.

Suitable "acyl" includes lower alkanoyl [e.g., formyl, acetyl, propionyl, hexanoyl, pivaloyl, etc.],
30 mono(or di or tri)halo(lower)alkanoyl [e.g., chloroacetyl, trifluoroacetyl, etc.], lower alkoxycarbonyl [e.g., methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl, tert-pentyloxycarbonyl, hexyloxycarbonyl, etc.], carbamoyl, aroyl [e.g., benzoyl, tolunoyl, naphthoyl, etc.], aryl(lower)alkanoyl [e.g., phenylacetyl, phenylpropionyl, etc.], aryloxycarbonyl
35 [e.g., phenoxycarbonyl, naphthyloxycarbonyl, etc.], aryloxy(lower)alkanoyl [e.g., phenoxyacetyl,

phenoxypropionyl, etc.], arylglyoxyloyl [e.g., phenylglyoxyloyl, naphthylglyoxyloyl, etc.], aryl(lower)alkoxycarbonyl which optionally substituted by suitable substituent(s) [e.g., benzyloxycarbonyl, phenethyloxycarbonyl, p-nitrobenzyloxycarbonyl, etc.], and the like.

Suitable "protected hydroxy" in the "protected hydroxy(lower)alkyl" includes acyloxy group, aryl(lower)alkyloxy group, and the like. Suitable "acyl" moiety in the "acyloxy" includes lower alkanoyl [e.g., formyl, acetyl, propionyl, hexanoyl, pivaloyl, etc.], mono(or di or tri)halo(lower)alkanoyl, [e.g., chloroacetyl, trifluoroacetyl, etc.], lower alkoxy carbonyl, [e.g., methoxycarbonyl, ethoxycarbonyl, tert-butoxycarbonyl, tert-pentyloxycarbonyl, hexyloxycarbonyl, etc.], carbamoyl, and the like.

Suitable "aryl(lower)alkyl" moiety in the "aryl(lower)alkyloxy" includes mono-, di- or triphenyl(lower)alkyl [e.g., benzyl, phenethyl, benzhydryl, trityl, etc.], and the like.

Suitable "protected carboxy" includes an esterified carboxy group and the like, and concrete examples of the ester moiety in said esterified carboxy group include the ones such as lower alkyl ester [e.g., methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, isobutyl ester, tert-butyl ester, pentyl ester, hexyl ester, 1-cyclopropylethyl ester, etc.] which may have suitable substituent(s), for example, lower alkanoyloxy(lower)alkyl ester [e.g., acetoxymethyl ester, propionyloxymethyl ester, butyryloxymethyl ester, valeryloxymethyl ester, pivaloyloxymethyl ester, 1-acetoxyethyl ester, 1-propionyloxyethyl ester, 2-propionyloxyethyl ester, hexanoyloxymethyl ester, etc.], lower alkanesulfonyl(lower)alkyl ester, [e.g., 2-mesyloxyethyl ester, etc.] or mono(or di or tri)halo(lower)alkyl ester [e.g., 2-iodoethyl ester, 2,2,2-trichloroethyl ester, etc.]; lower alkenyl ester [e.g., vinyl ester, allyl ester, etc.]; lower alkynyl

ester [e.g., ethynyl ester, propynyl ester, etc.]; aryl(lower)alkyl ester which may have suitable substituent(s) [e.g., benzyl ester, 4-methoxybenzyl ester, 4-nitrobenzyl ester, phenethyl ester, trityl ester, 5 benzhydryl ester, bis(methoxyphenyl)methyl ester, 3,4-dimethoxybenzyl ester, 4-hydroxy-3,5-di-tert-butylbenzyl ester, etc.]; aryl ester which may have suitable substituent(s) [e.g., phenyl ester, 4-chlorophenyl ester, tolyl ester, 4-tert-butylphenyl ester, xylyl ester, 10 mesityl ester, cumenyl ester, etc.]; and the like.

Suitable "leaving group" includes halogen [e.g., chlorine, bromine, iodine, etc.] or acyloxy such as arylsulfonyloxy [e.g., benzenesulfonyloxy, tosyloxy, etc.], lower alkylsulfonyloxy [e.g., mesyloxy, etc.], 15 lower alkanoyloxy [e.g., acetyloxy, propionyloxy, etc.], and the like.

Suitable "anion" includes formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate, toluenesulfonate, chloride, bromide, 20 iodide, sulfate, hydrogensulfate, phosphate, and the like.

Suitable pharmaceutically acceptable salts of the object compound [I] are conventional non-toxic salts and include, for example, a salt with a base or an acid 25 addition salt such as a salt with an inorganic base, for example, an alkali metal salt [e.g., sodium salt, potassium salt, etc.], an alkaline earth metal salt [e.g., calcium salt, magnesium salt, etc.], an ammonium salt; a salt with an organic base, for example, an 30 organic amine salt [e.g., trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, etc.]; an inorganic acid addition salt [e.g., 35 hydrochloride, hydrobromide, sulfate, hydrogensulfate, phosphate, etc.]; an organic carboxylic or sulfonic acid addition salt [e.g., formate, acetate, trifluoroacetate, maleate, tartrate, citrate, fumarate, methanesulfonate,

benzenesulfonate, toluenesulfonate, etc.]; and a salt with a basic or acidic amino acid [e.g., arginin , aspartic acid, glutamic acid, etc.].

5 The preferred embodiments of the cephem compound of the present invention represented by the general formula [I] are as follows.

(1) The compound of the formula [I] wherein
R¹ is lower alkyl or hydroxy(lower)alkyl, and
R² is hydrogen, aryl(lower)alkyl or acyl, or
10 R¹ and R² are bonded together and form lower alkylene;
R⁴ is carboxy or esterified carboxy;
R⁵ is amino or acylamino;
R¹¹ is hydrogen or acyl; and
R¹² is amino or acylamino,

15 or a pharmaceutically acceptable salt thereof.

(2) The compound of (1) above wherein
R¹ is lower alkyl or hydroxy(lower)alkyl, and
R² is hydrogen, aryl(lower)alkyl, lower alkanoyl or
lower alkoxy carbonyl, or
20 R¹ and R² are bonded together and form lower alkylene;
R⁴ is carboxy or lower alkoxy carbonyl;
R⁵ is amino, lower alkanoylamino or lower
alkoxy carbonylamino;
R¹¹ is hydrogen, lower alkanoyl or lower alkoxy carbonyl;
25 and
R¹² is amino, lower alkanoylamino or lower
alkoxy carbonylamino,

or a pharmaceutically acceptable salt thereof.

(3) The compound of (2) above wherein
30 R¹ is (C₁-C₆)alkyl or hydroxy(C₁-C₆)alkyl, and
R² is hydrogen, mono-, di- or triphenyl(C₁-C₆)alkyl,
(C₁-C₆)alkanoyl or (C₁-C₆)alkoxy carbonyl, or
R¹ and R² are bonded together and form (C₁-C₆)alkylene;
R⁴ is carboxy or (C₁-C₆)alkoxy carbonyl;
35 R⁵ is amino, (C₁-C₆)alkanoylamino or
(C₁-C₆)alkoxy carbonylamino;
R¹¹ is hydrogen, (C₁-C₆)alkanoyl or (C₁-C₆)alkoxy carbonyl;
and

R¹² is amino, (C₁-C₆)alkanoylamino or
(C₁-C₆)alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

(4) The compound of (2) above wherein

- 5 R¹ is lower alkyl or hydroxy(lower)alkyl, and
R² is hydrogen, or
R¹ and R² are bonded together and form lower alkylene;
R⁴ is carboxy;
R⁵ is amino;
10 R¹¹ is hydrogen or lower alkanoyl; and
R¹² is amino,

or a pharmaceutically acceptable salt thereof.

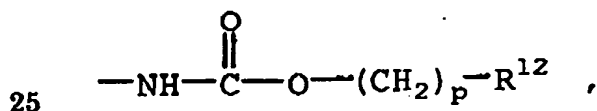
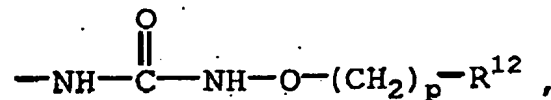
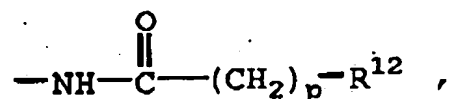
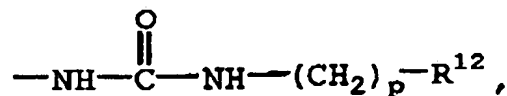
(5) The compound of (4) above wherein

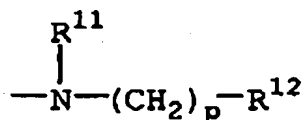
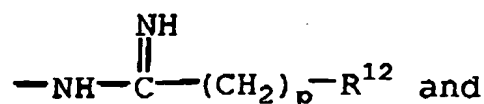
- R¹ is (C₁-C₆)alkyl or hydroxy(C₁-C₆)alkyl, and
15 R² is hydrogen, or
R¹ and R² are bonded together and form (C₁-C₆)alkylene;
R⁴ is carboxy;
R⁵ is amino;
R¹¹ is hydrogen or (C₁-C₆)alkanoyl; and
20 R¹² is amino,

or a pharmaceutically acceptable salt thereof.

(6) The compound of the formula [I] wherein

R³ is selected from the group consisting of





wherein R^{11} , R^{12} and p are each as defined above,
or a pharmaceutically acceptable salt thereof.

(7) The compound of (6) above wherein

5 R^{11} is hydrogen, lower alkanoyl or lower alkoxy carbonyl;
and

R^{12} is amino, lower alkanoylamino or lower
alkoxy carbonylamino,

or a pharmaceutically acceptable salt thereof.

10 (8) The compound of (7) above wherein

R^{11} is hydrogen, (C_1-C_6) alkanoyl or (C_1-C_6) alkoxy carbonyl;
and

R^{12} is amino, (C_1-C_6) alkanoylamino or
 (C_1-C_6) alkoxy carbonylamino,

15 or a pharmaceutically acceptable salt thereof.

(9) The compound of (7) above wherein

R^{11} is hydrogen or lower alkanoyl; and

R^{12} is amino,

or a pharmaceutically acceptable salt thereof.

20 (10) The compound of (9) above wherein

R^{11} is hydrogen or (C_1-C_6) alkanoyl; and

R^{12} is amino,

or a pharmaceutically acceptable salt thereof.

25 The processes for preparing the object compound of
the present invention are explained in detail in the
following.

Process 1

30 The compound [I] or a salt thereof can be prepared
by reacting the compound [II] or its reactive derivative
at the amino group, or a salt thereof with the compound
[III] or its reactive derivative at the carboxy group,
or a salt thereof.

Suitable reactive derivative at the amino group of the compound [II] includes Schiff's base type imino or its tautomeric enamine type isomer formed by the reaction of the compound [II] with a carbonyl compound such as aldehyde, ketone and the like; a silyl derivative formed by the reaction of the compound [II] with a silyl compound such as bis(trimethylsilyl)acetamide, mono(trimethylsilyl)acetamide [e.g., N-(trimethylsilyl)acetamide], bis(trimethylsilyl)urea and the like; a derivative formed by the reaction of the compound [II] with phosphorus trichloride or phosgene.

Suitable salts of the compound [II] and its reactive derivative can be referred to the ones as exemplified for the compound [I].

Suitable reactive derivative at the carboxy group of the compound [III] includes an acid halide, an acid anhydride, an activated amide, and an activated ester. A suitable example of the reactive derivatives may be an acid chloride; an acid azide; a mixed acid anhydride with an acid such as substituted phosphoric acid [e.g., dialkylphosphoric acid, phenylphosphoric acid, diphenylphosphoric acid, dibenzylphosphoric acid, halogenated phosphoric acid, etc.], dialkylphosphorous acid, sulfurous acid, thiosulfuric acid, sulfuric acid, alkanesulfonic acid [e.g., methanesulfonic acid, etc.], aliphatic carboxylic acid [e.g., acetic acid, propionic acid, butyric acid, isobutyric acid, pivalic acid, pentanoic acid, isopentanoic acid, 2-ethylbutyric acid, trichloroacetic acid, etc.] and aromatic carboxylic acid [e.g., benzoic acid, etc.]; a symmetrical acid anhydride; an activated amide with imidazole, 4-substituted imidazole, dimethylpyrazole, triazole or tetrazole; an activated ester [e.g., cyanomethyl ester, methoxymethyl ester, dimethyliminomethyl $[(CH_3)_2N^+=CH-]$ ester, vinyl ester, propargyl ester, p-nitrophenyl ester, 2,4-dinitrophenyl ester, trichlorophenyl ester, pentachlorophenyl ester, mesylphenyl ester,

phenylazophenyl ester, phenyl thioester, p-nitrophenyl thioester, p-cresyl thioester, carboxymethyl thio ester, pyranyl ester, pyridyl ester, piperidyl ester, 8-quinolyl thioester, etc.); or an ester with an N-hydroxy compound [e.g., N,N-dimethylhydroxylamine, 1-hydroxy-2-(1H)-pyridone, N-hydroxysuccinimide, N-hydroxyphthalimide, N-hydroxy-1H-benzotriazole, etc.]. These reactive derivatives can optionally be selected from them according to the kind of the compound [III] to be used.

Suitable salts of the compound [III] and its reactive derivative can be referred to the ones as exemplified for the compound [I].

The reaction is usually carried out in a conventional solvent such as water, alcohol [e.g., methanol, ethanol, etc.], acetone, dioxane, acetonitrile, chloroform, methylene chloride, ethylene chloride, tetrahydrofuran, ethyl acetate, N,N-dimethylformamide, pyridine or any other organic solvent which does not adversely affect the reaction. These conventional solvents may also be used in a mixture with water.

In this reaction, when the compound [III] is used in free acid form or its salt form, the reaction is preferably carried out in the presence of a conventional condensing agent such as N,N'-dicyclohexylcarbodiimide; N-cyclohexyl-N'-morpholinoethylcarbodiimide; N-cyclohexyl-N'-(4-diethylaminocyclohexyl)carbodiimide; N,N'-diethylcarbodiimide; N,N'-diisopropylcarbodiimide; N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide; N,N'-carbonyl-bis-(2-methylimidazole); pentamethyleneketene-N-cyclohexylimine; diphenylketene-N-cyclohexylimine; ethoxyacetylene; 1-alkoxy-1-chloroethylene; trialkyl phosphite; ethyl polyphosphate; isopropyl polyphosphate; phosphorus oxychloride (phosphoryl chloride); phosphorus trichloride; thionyl chloride; oxalyl chloride; lower alkyl haloformate [e.g., ethyl chloroformate, isopropyl chloroformate, etc.], triphenylphosphine; 2-ethyl-7-hydroxybenzisoxazolium salt; 2-ethyl-5-(m-

sulfoph nyl)isoxazolium hydroxide intramolecular salt;
1-(p-chlorobenzenesulfonyloxy)-6-chloro-1H-
benzotriazole; so-called Vilsmeier reagent prepared by
the reaction of N,N-dimethylformamide with thionyl
5 chloride, phosgene, trichloromethyl chloroformate,
phosphorus oxychloride, etc.; and the like.

The reaction may also be carried out in the
presence of an inorganic or organic base such as an
alkali metal bicarbonate, tri(lower)alkylamine, pyridine,
10 N-(lower)alkylmorpholine, N,N-di(lower)alkylbenzylamine,
and the like.

The reaction temperature is not critical, and the
reaction is usually carried out under cooling to warming.

Process 2

15 The compound [Ib] or a salt thereof can be
prepared by subjecting the compound [Ia] or a salt
thereof to elimination reaction of the amino protecting
group.

Elimination reaction is carried out in accordance
20 with a conventional method such as hydrolysis and the
like.

The hydrolysis is preferably carried out in the
presence of a base or an acid including Lewis acid.

Suitable base includes an inorganic base and an
25 organic base such as an alkali metal [e.g., sodium,
potassium, etc.], an alkaline earth metal [e.g.,
magnesium, calcium, etc.], the hydroxide or carbonate or
hydrogencarbonate thereof, trialkylamine [e.g.,
trimethylamine, triethylamine, etc.], picoline, 1,5-
30 diazabicyclo[4.3.0]non-5-ene, 1,4-
diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-
7-ene, and the like.

Suitable acid includes an organic acid [e.g.,
formic acid, acetic acid, propionic acid,
35 trichloroacetic acid, trifluoroacetic acid, etc.], and
an inorganic acid [e.g., hydrochloric acid, hydrobromic
acid, sulfuric acid, hydrogen chloride, hydrogen bromide,
etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g., trichloroacetic acid, trifluoroacetic acid, etc.], and the like is preferably carried out in the presence of cation trapping agents
5 [e.g., anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, alcohol [e.g., methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence
10 the reaction. A liquid base or acid can be also used as a solvent.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.
Process 3-(i)

15 The compound [VIII] or a salt thereof can be prepared by reacting the compound [VI] or a salt thereof with the compound [VII] or a salt thereof.

Suitable salt of the compounds [VI], [VII] and [VIII] can be referred to the ones as exemplified for
20 the compound [I].

The present reaction may be carried out in a solvent such as water, phosphate buffer, acetone, chloroform, acetonitrile nitrobenzene, methylene chloride, ethylene chloride, formamide, N,N-
25 dimethylformamide, methanol, ethanol, diethyl ether, tetrahydrofuran, dimethyl sulfoxide, or any other organic solvent which does not adversely affect the reaction, preferably in ones having strong polarities. Among the solvents, hydrophilic solvents may be used in
30 a mixture with water. When the compound [VII] is liquid, it can also be used as a solvent.

The reaction is preferably conducted in the presence of a base, for example, an inorganic base such as alkali metal hydroxide, alkali metal carbonate,
35 alkali metal hydrogencarbonate, an organic base such as trialkylamine, and the like.

The reaction temperature is not critical, and the reaction is usually carried out at ambient temperature,

under warming or under heating. The present reaction is preferably carried out in the presence of alkali metal halide [e.g., sodium iodide, potassium iodide, etc.], alkali metal thiocyanate [e.g., sodium thiocyanate, potassium thiocyanate, etc.], and the like.

Anion X^{\ominus} may be one derived from a leaving group Y, and it may be converted to other anion by a conventional method.

Process 3-(ii)

The compound [I] or a salt thereof can be prepared by subjecting the compound [VIII] or a salt thereof to elimination reaction of the carboxy protecting group.

Elimination reaction is carried out in similar manner to the reaction in the aforementioned Process 2, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 2.

Process 4

The compound [Id] or a salt thereof can be prepared by subjecting the compound [Ic] or a salt thereof to elimination reaction of the hydroxy protecting group.

Suitable method of this elimination reaction includes conventional one such as hydrolysis, reduction and the like.

(i) For hydrolysis:

The hydrolysis is preferably carried out in the presence of a base or an acid including Lewis acid.

Suitable base includes an inorganic base and an organic base such as an alkali metal [e.g., sodium, potassium, etc.], an alkaline earth metal [e.g., magnesium, calcium, etc.], the hydroxide or carbonate or hydrogencarbonate thereof, trialkylamine [e.g., trimethylamine, triethylamine, etc.], picoline, 1,5-diazabicyclo[4.3.0]non-5-ene, 1,4-diazabicyclo[2.2.2]octane, 1,8-diazabicyclo[5.4.0]undec-7-ene, and the like.

Suitable acid includes an organic acid [e.g.,

formic acid, acetic acid, propionic acid, trichloroacetic acid, trifluoroacetic acid, etc.], and an inorganic acid [e.g., hydrochloric acid, hydrobromic acid, sulfuric acid, hydrogen chloride, hydrogen bromide, etc.].

The elimination using Lewis acid such as trihaloacetic acid [e.g., trichloroacetic acid, trifluoroacetic acid, etc.] and the like is preferably carried out in the presence of cation trapping agents [e.g., anisole, phenol, etc.].

The reaction is usually carried out in a solvent such as water, alcohol [e.g., methanol, ethanol, etc.], methylene chloride, tetrahydrofuran, a mixture thereof or any other solvent which does not adversely influence the reaction. A liquid base or acid can be also used as a solvent.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming. (ii) For reduction:

Reduction is carried out in a conventional manner, including chemical reduction and catalytic reduction.

Suitable reducing reagents to be used in chemical reduction are a combination of a metal [e.g., tin, zinc, iron, etc.] or metallic compound [e.g., chromium chloride, chromium acetate, etc.] and an organic acid or inorganic acid [e.g., formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.].

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts [e.g., platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.], palladium catalysts [e.g., spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.], nickel catalysts [e.g., reduced nickel, nickel oxide, Raney nickel, etc.], cobalt catalysts [e.g., reduced cobalt,

Raney cobalt, etc.], iron catalysts [e.g., reduced iron, Raney iron, etc.], copper catalysts [e.g., reduced copper, Raney copper, Ullman copper, etc.] and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, methanol, ethanol, propanol, N,N-dimethylformamide or a mixture thereof.

Additionally, in case that the above-mentioned acids to be used in chemical reduction are liquid, they can also be used as a solvent.

Further, a suitable solvent to be used in catalytic reduction may be the above-mentioned solvent, and other conventional solvent such as diethyl ether, dioxane, tetrahydrofuran, etc., or a mixture thereof.

The reaction temperature of this reduction is not critical and the reaction is usually carried out under cooling to warming.

When R⁵ is protected amino, the amino protecting group in R⁵ can be eliminated by a conventional method such as hydrolysis.

Processes A and B for the preparation of the starting compounds are explained in detail in the following.

Process A-(i)

The compound [XII] or a salt thereof can be prepared by reacting the compound [X] or a salt thereof with the compound [XI] or a salt thereof.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Process 3-(i), and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 3-(i).

Process A-(ii)

The compound [II] or a salt thereof can be prepared by subjecting the compound [XII] or a salt thereof to elimination reaction of the amino protecting groups in R⁸ and R¹⁰ and the carboxy protecting group in R⁹.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Proc ss 2, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 2.

Process B

The compound [VI] or a salt thereof can be prepared by reacting the compound [XIII] or its reactive derivative at the amino group, or a salt thereof with the compound [XIV] or its reactive derivative at the carboxy group, or a salt thereof.

This reaction can be carried out in a similar manner to the reaction in the aforementioned Process 1, and therefore the reagents to be used and reaction conditions (e.g., solvent, reaction temperature, etc.) can be referred to those of Process 1.

The compounds obtained by the above processes can be isolated and purified by a conventional method such as pulverization, recrystallization, column chromatography, reprecipitation, and the like.

It is to be noted that the compound [I] and other compounds may include one or more stereoisomer(s) such as optical isomer(s) and geometrical isomer(s) due to asymmetric carbon atom(s) and double bond(s), and all of such isomers and mixtures thereof are included within the scope of this invention.

The object compounds [I] and pharmaceutically acceptable salts thereof include solvates [e.g., enclosure compounds (e.g., hydrate, etc.)].

The object compound [I] and pharmaceutically acceptable salts thereof are novel and exhibit high antimicrobial activity, inhibiting the growth of a wide variety of pathogenic microorganisms including Gram-positive and Gram-negative microorganisms and are useful as antimicrobial agents.

Now in order to show the utility of the object compound [I], the test data on MIC (minimal inhibitory concentration) of a representative compound of this

invention are shown in the following.

Test method:

In vitro antibacterial activity was determined by the two-fold agar-plate dilution method as described below.

One loopful of an overnight culture of each test strain in Trypticase-soy broth (10^6 viable cells per ml) was streaked on heart infusion agar (HI-agar) containing graded concentrations of representative test compound, and the minimal inhibitory concentration (MIC) was expressed in $\mu\text{g/ml}$ after incubation at 37°C for 20 hours.

Test compound

Compound (a): 7β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(3-aminopropionamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (Example 3)

Compound (b): 7β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionamido)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (Example 4)

Compound (c): 7β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate hydrogen sulfate (Example 6)

Compound (d): 7β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate hydrogen sulfate (Example 7)

Compound (e): 7β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate hydrogen sulfate (Example 11)

Ceftazidime

Test results:

Table 1

Test strain	Test compound	MIC ($\mu\text{g/ml}$)
<i>Pseudomonas aeruginosa</i> FP 1380	(a)	2
	(b)	1
	(c)	2
	(d)	2
	(e)	1
	Ceftazidime	128

For therapeutic administration, the object
5 compound [I] and pharmaceutically acceptable salts
thereof of the present invention are used in the form of
a conventional pharmaceutical preparation which contains
said compound as an active ingredient, in admixture with
pharmaceutically acceptable carriers such as an organic
10 or inorganic solid or liquid excipient which is suitable
for oral, parenteral or external administration. The
pharmaceutical preparations may be in a solid form such
as tablet, granule, powder, capsule, or in a liquid form
such as solution, suspension, syrup, emulsion, lemonade
15 and the like.

If needed, there may be included in the above
preparations auxiliary substances, stabilizing agents,
wetting agents and other commonly used additives such as
lactose, citric acid, tartaric acid, stearic acid,
20 magnesium stearate, terra alba, sucrose, corn starch,
talc, gelatin, agar, pectin, peanut oil, olive oil,
cacao butter, ethylene glycol, and the like.

While the dosage of the compound [I] may vary from
and also depend upon the age, conditions of the patient,
25 a kind of diseases, a kind of the compound [I] to be
applied, etc. In general amounts between 1 mg and 4,000
mg or even more per day may be administered to a patient.
An average single dose of about 50 mg, 100 mg, 250 mg,
500 mg, 1000 mg or 2000 mg of the object compounds [I]
30 of the present invention may be used in treating
diseases infected by pathogenic microorganisms.

The following Preparations and Examples are given

for the purpose of illustrating the present invention in more detail.

Preparation 1

To a solution of (Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)[(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (5 g) in a mixture of tetrahydrofuran (80 ml) and N,N-dimethylformamide (20 ml) was added a solution of sodium bis(trimethylsilyl)amide (8.33 g) in tetrahydrofuran (12 ml), and the mixture was stirred for 15 minutes. To the reaction mixture was added a solution of di-tert-butyl dicarbonate (3.3 g) in tetrahydrofuran (20 ml) under ice-cooling, and the mixture was stirred under ice-cooling for 3 hours. To the reaction mixture was added ethyl acetate, and the mixture was washed with 10% aqueous potassium hydrogen sulfate solution, and then washed with a phosphate buffer (pH 6.86). The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether and dried in vacuo to give (Z)-2-[5-[(tert-butoxycarbonyl)amino]-1,2,4-thiadiazol-3-yl][(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (3.10 g).
IR(KBr) 3191.6, 2981.4, 1714.4, 1550.5, 1153.2, 1000.9 cm^{-1}

$^1\text{H-NMR}$ (DMSO- d_6) δ 1.37 (9H, s), 1.45 (6H, s), 1.50 (9H, s), 12.7 (1H, s)

ESI-MS: $m/z=429$ (M-H)

Preparation 2

A mixture of N,N-dimethylformamide (0.648 ml) and phosphoryl chloride (0.781 ml) was stirred at room temperature for 30 minutes. To the mixture were added tetrahydrofuran (4 ml) and (Z)-2-[5-[(tert-butoxycarbonyl)amino]-1,2,4-thiadiazol-3-yl][(2-tert-butoxy-1,1-dimethyl-2-oxoethoxy)imino]ethanoic acid (3 g) at 4°C, and the reaction mixture was stirred at room temperature for 1 hour. Meanwhile, a mixture of benzhydryl 7 β -amino-3-chloromethyl-3-cephem-4-

carboxylate hydrochloride (3 g) and N-trimethylsilylacetamide (8.72 g) in tetrahydrofuran (15 ml) was warmed to make a clear solution. The solution was then cooled to -20°C and added to the activated acid solution obtained above. The reaction mixture was stirred at a temperature of -10°C to 0°C for 1 hour and poured into a mixture of ethyl acetate and water. The aqueous layer was separated, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo and purified by column chromatography on silica gel eluting with hexane/ethyl acetate (3:2) to give benzhydryl 7β-[(2)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (4.79 g).

IR(KBr) 2981.4, 1793.5, 1720.2, 1524.8, 1371.1, 1247.7, 1151.3 cm^{-1}

$^1\text{H-NMR}$ (DMSO- d_6) δ 1.39 (6H, s), 1.48 (3H, s), 1.50 (6H, s), 3.58 (1H, d, $J=18.3\text{Hz}$), 3.76 (1H, d, $J=18.3\text{Hz}$), 4.44 (2H, s), 5.29 (1H, d, $J=5.0\text{Hz}$), 6.01 (1H, dd, $J=8.6, 5.0\text{Hz}$), 6.97 (1H, s), 7.2-7.6 (10H, m), 9.65 (1H, d, $J=5.0\text{Hz}$), 12.7 (1H, s)

ESI-MS: $m/z=849$ (M+Na)

25 Preparation 3

To a solution of 5-amino-1-methylpyrazole (5 g) in ethanol (50 ml) was added isoamyl nitrite (6.92 ml) and then 20% hydrochloric acid (5 drops) was added at 4°C. The reaction mixture was refluxed for 2 hours and cooled to room temperature. To the reaction mixture was added diisopropyl ether (50 ml) and the mixture was stirred for 0.5 hour. The resulting precipitate was collected by filtration and dried in vacuo to give 5-amino-1-methyl-4-nitrosopyrazole (3.53 g, yield 54.4 %).

35 $^1\text{H-NMR}$ (DMSO- d_6) δ 3.51 (3H, s), 8.07 (2H, brs), 8.51 (1H, s)

AP-MS: $m/z=127$ (M+H)

Preparation 4

To a solution of 5-amino-1-methyl-4-nitrosopyrazole (1 g) in water (40 ml) were added concentrated sulfuric acid (0.423 ml) and palladium on carbon (0.3 g) under a hydrogen atmosphere. The mixture
5 was stirred overnight. The reaction mixture was filtered and the filtrate was evaporated in vacuo. To the residue was added isopropyl alcohol and the resulting precipitate was collected by filtration to give 4,5-diamino-1-methylpyrazole sulfuric acid salt
10 (1.71 g, quantitative yield).

$^1\text{H-NMR}$ (DMSO- d_6) δ 3.54 (3H, s), 7.19 (1H, s)

ESI-MS: $m/z=113$ (M+H)

Preparation 5

To a suspension of 1,1'-carbonyldiimidazole (9.73
15 g, 60 mmol) in dehydrated chloroform (72 ml) was added tert-butyl N-(2-aminoethyl)carbamate (9.61 g, 60 mmol) under ice-cooling, and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyldiisopropylamine (14.22 g, 110 mmol) and
20 4,5-diamino-1-methylpyrazole sulfuric acid salt (10.51 g, 50 mmol); and the mixture was stirred at 50°C for 15 hours. The insoluble materials were removed by filtration. To the filtrate were added chloroform (200
25 ml) and 5% aqueous sodium hydrogen carbonate solution (100 ml). The organic layer was separated and the aqueous layer was extracted with a mixed solvent of chloroform and methanol (4:1). The organic layers were combined, dried over anhydrous magnesium sulfate,
30 filtered, and concentrated in vacuo. The residue was triturated with ethyl acetate and dried in vacuo to give 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}-ureido)-1-methylpyrazole (14.0 g) as a solid.

$^1\text{H-NMR}$ (DMSO- d_6) δ 1.38 (9H, s), 2.96-2.98 (2H, m), 3.03-
3.07 (2H, m), 3.50 (3H, s), 4.81 (2H, br), 5.92 (1H, br),
35 6.80 (1H, br), 6.96 (1H, s), 7.18 (1H, br)

Example 1

To a solution of benzhydryl 7 β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-

butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (500 mg, 0.60 mmol) in N,N-dimethylformamide (1.0 ml) was added sodium iodide (100 mg, 0.66 mmol), and the mixture was stirred
5 at room temperature for 30 minutes. To the reaction mixture was added a solution of 5-amino-4-(3-(2-[(tert-butoxycarbonyl)amino]ethyl)ureido)-1-methylpyrazole (216 mg, 0.73 mmol) in N,N-dimethylformamide (1.0 ml). The whole mixture was stirred at 32°C for 4 hours. To the
10 resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was
15 concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (75 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (1.8 ml) were added anisole (0.6 ml)
20 and trifluoroacetic acid (1.2 ml). The resulting solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (80 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (380 mg), which
25 was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and
30 chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-
35 pyrazolio)methyl-3-cephem-4-carboxylate (21 mg) as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 3.12 (2H, t,

J=5.7Hz), 3.22 (1H, d, J=17.9Hz), 3.49 (1H, d, J=17.9Hz), 3.46 (2H, t, J=5.7Hz), 3.71 (3H, s), 4.95 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.6Hz), 5.84 (1H, d, J=4.6Hz), 7.89 (1H, s)

5 Preparation 6

To a solution of 5-amino-4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-1-methylpyrazole (597 mg, 2 mmol) and triethylamine (243 mg, 2.4 mmol) in methylene chloride (10 ml) was added triphenylmethyl chloride (669 mg, 2.4 mmol), and the mixture was stirred at room temperature for 19 hours. The reaction mixture was washed successively with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with ethyl acetate to give 4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}-ureido)-1-methyl-5-triphenylmethylaminopyrazole (640 mg) as a solid.

¹H-NMR(DMSO-d₆) δ 1.38 (9H, s), 2.70 (3H, s), 2.94-2.96 (2H, m), 2.99-3.01 (2H, m), 5.68 (1H, brs), 5.96 (1H, br), 6.78 (1H, br), 6.85 (1H, br), 7.00 (1H, s), 7.13-7.15 (6H, m), 7.24-7.28 (9H, m)

Example 2

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (810 mg, 1.0 mmol) in N,N-dimethylformamide (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added a solution of 4-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-1-methyl-5-triphenylmethylaminopyrazole (640 mg, 1.2 mmol) in methylene chloride (10 ml). The whole mixture was stirred at room temperature for 26 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated,

and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate
5 was poured into diisopropyl ether (80 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (2.38 ml) were added anisole (0.79 ml) and trifluoroacetic acid (1.58 ml). The resulting
10 solution was stirred at room temperature for 4 hours and poured into diisopropyl ether (80 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (635 mg), which was purified by preparative high-performance liquid
15 chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical
20 Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate (54 mg) as an
25 amorphous solid.
¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 3.12 (2H, t, J=5.7Hz), 3.22 (1H, d, J=17.9Hz), 3.49 (1H, d, J=17.9Hz), 3.46 (2H, t, J=5.7Hz), 3.71 (3H, s), 4.95 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.6Hz),
30 5.84 (1H, d, J=4.6Hz), 7.89 (1H, s)

Preparation 7

To a solution of 2,3-dihydro-1H-imidazo[1,2-b]pyrazole (120 g, 1.1 mol) in sulfuric acid (500 ml)
35 was added potassium nitrate (111 g, 1.1 mol) under ice-cooling. The mixture was stirred at room temperature for 48 hours. The reaction mixture was added to ice (2.0 kg). The crystalline residue was collected by

filtration and dried in vacuo to give 7-nitro-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (132 g) as a solid.

¹H-NMR(DMSO-d₆) δ 4.05-4.09 (2H, m), 4.17-4.20 (2H, m), 7.82 (1H, s), 7.97 (1H, br)

5 Preparation 8

A suspension of 7-nitro-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (97 g, 629 mmol) in a mixed solvent of sulfuric acid (34 ml) and water (2000 ml) was treated with 10% palladium on carbon (10 g) under a hydrogen atmosphere at room temperature for 4 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with methanol and dried in vacuo to give 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (90.2 g) as a solid.

¹H-NMR(DMSO-d₆) δ 3.87-3.90 (2H, m), 4.07-4.10 (2H, m), 7.28 (1H, s)

Preparation 9

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (2.22 g, 10 mmol) and N-ethyldiisopropylamine (2.84 g, 22 mmol) in methylene chloride (70 ml) was added N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (3.15 g, 11 mmol). The mixture was stirred at room temperature for 4 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution and the organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 7-[3-(tert-butoxycarbonylamino)propionyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (2.2 g) as a solid.

¹H-NMR(CDCl₃) δ 1.44 (9H, s), 2.52 (2H, t, J=6.0Hz), 3.36-3.47 (2H, m), 3.96 (2H, t, J=8.2Hz), 4.18 (2H, t, J=8.2Hz), 5.16 (1H, br), 7.16 (1H, s), 7.90 (1H, br)

Example 3

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(3-

aminopropionamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 7-[3-(tert-butoxycarbonylamino)propionyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole in the same manner as in Example 1 as an amorphous solid.

¹H-NMR(D₂O) δ 1.51 (3H, s), 1.52 (3H, s), 2.83 (2H, t, J=6.4Hz), 3.26 (1H, d, J=17.9Hz), 3.53 (1H, d, J=17.9Hz), 3.31 (2H, t, J=6.4Hz), 4.15 (2H, t, J=8.7Hz), 4.33 (1H, q, J=8.7Hz), 4.42 (1H, q, J=8.7Hz), 4.95 (1H, d, J=15.1Hz), 5.03 (1H, d, J=15.1Hz), 5.25 (1H, d, J=5.0Hz), 5.84 (1H, d, J=5.0Hz), 8.06 (1H, s)

Preparation 10

tert-Butyl [2-(5-amino-1-methyl-1H-pyrazol-4-ylcarbamoyl)ethyl]carbamate

The title compound was obtained from 4,5-diamino-1-methylpyrazole sulfuric acid salt and N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide in the same manner as in Preparation 9.

¹H-NMR(DMSO-d₆) δ 1.38 (9H, s), 2.35 (2H, t, J=7.1Hz), 3.18 (2H, q, J=7.1Hz), 3.50 (3H, s), 4.90 (2H, s), 6.83 (1H, t, J=7.1Hz), 7.14 (1H, s), 9.06 (1H, s)

AP-MS: m/z=283

Preparation 11

tert-Butyl {2-[1-methyl-5-(tritylamino)-1H-pyrazol-4-ylcarbamoyl]ethyl}carbamate

The title compound was obtained from tert-butyl [2-(5-amino-1-methyl-1H-pyrazol-4-ylcarbamoyl)ethyl]carbamate in the same manner as in Preparation 6.

¹H-NMR(DMSO-d₆) δ 1.39 (9H, s), 2.08 (2H, t, J=7.1Hz), 2.71 (3H, s), 3.04 (2H, q, J=7.1Hz), 5.57 (1H, s), 6.72 (1H, t, J=7.1Hz), 7.1-7.4 (16H, m), 8.25 (1H, s)

Example 4

7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-

aminopropionamido)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7 β -[(2)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and tert-butyl (2-([1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]carbamoylethyl)carbamate in the same manner as in Example 1.

¹H-NMR (D₂O) δ 1.53 (6H, s), 2.89 (2H, t, J=6.5Hz), 3.20 and 3.47 (2H, ABq, J=18Hz), 3.34 (2H, t, J=6.5Hz), 3.75 (3H, s), 4.99 and 5.21 (2H, ABq, J=16Hz), 5.25 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.02 (1H, s)
ESI-MS: m/z=674 (M+Na)

15 Preparation 12

To a solution of 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethylsulfonyl)guanidine (22.3 g) in dichloromethane (100 ml) were added 4,5-diamino-1-methylpyrazole sulfuric acid salt (10 g) and triethylamine (33.2 ml) at 4°C, and the mixture was stirred at room temperature overnight. The reaction mixture was poured into a mixture of ethyl acetate and water. The aqueous layer was separated, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo. The concentrate was poured into acetonitrile, and the resulting precipitate was collected by filtration and dried in vacuo to give 5-amino-4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methylpyrazole (11.62 g, yield 68.9%).

¹H-NMR (DMSO-d₆) δ 1.37 (9H, s), 1.50 (9H, s), 3.52 (3H, s), 5.14 (2H, s), 7.11 (1H, s), 9.14 (1H, s), 11.5 (1H, s)
ESI-MS: m/z=353 (M-H)

35 Preparation 13

4-[2',3'-Bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-(tritylamino)pyrazole

The title compound was obtained from 5-amino-4-

aminopropionamido)-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and tert-butyl (2-((1-methyl-5-(tritylamino)-1H-pyrazol-4-yl)carbamoyl)ethyl)carbamate in the same manner as in Example 1.

¹H-NMR(D₂O) δ 1.53 (6H, s), 2.89 (2H, t, J=6.5Hz), 3.20 and 3.47 (2H, ABq, J=18Hz), 3.34 (2H, t, J=6.5Hz), 3.75 (3H, s), 4.99 and 5.21 (2H, ABq, J=16Hz), 5.25 (1H, d, J=4.8Hz), 5.85 (1H, d, J=4.8Hz), 8.02 (1H, s)
ESI-MS: m/z=674(M+Na)

15 Preparation 12

To a solution of 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethylsulfonyl)guanidine (22.3 g) in dichloromethane (100 ml) were added 4,5-diamino-1-methylpyrazole sulfuric acid salt (10 g) and triethylamine (33.2 ml) at 4°C, and the mixture was stirred at room temperature overnight. The reaction mixture was poured into a mixture of ethyl acetate and water. The aqueous layer was separated, and the organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo. The concentrate was poured into acetonitrile, and the resulting precipitate was collected by filtration and dried in vacuo to give 5-amino-4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methylpyrazole (11.62 g, yield 68.9%).

¹H-NMR(DMSO-d₆) δ 1.37 (9H, s), 1.50 (9H, s), 3.52 (3H, s), 5.14 (2H, s), 7.11 (1H, s), 9.14 (1H, s), 11.5 (1H, s)

ESI-MS: m/z=353(M-H)

35 Preparation 13

4-[2',3'-Bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-(tritylamino)pyrazole

The title compound was obtained from 5-amino-4-

[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methylpyrazole in the same manner as in Preparation 6.

¹H-NMR(DMSO-d₆) δ 1.37 (9H, s), 1.50 (9H, s), 2.85 (3H, s), 5.88 (1H, s), 7.17 (1H, s), 7.21 (15H, m), 8.85 (1H, s), 11.2 (1H, s)

ESI-MS: m/z=597 (M+H).

Example 5

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate.

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-(tritylamino)pyrazole in the same manner as in Example 1.

¹H-NMR(DMSO-d₆) δ 1.53 (6H, s), 3.25 and 3.57 (2H, ABq, J=18Hz), 3.75 (3H, s), 5.00 and 5.18 (2H, ABq, J=15Hz), 5.27 (1H, d, J=4.9Hz), 5.85 (1H, d, J=4.9Hz), 8.05 (1H, s)

Preparation 14

To a suspension of 4,5-diamino-1-methylpyrazole sulfuric acid salt (305 g, 1.45 mol) in tetrahydrofuran (3.05 L) was added tert-butyl 2-[(2,5-dioxo-1-pyrrolidinyl)oxyl]-2-oxoethylcarbamate (415 g, 1.52 mol) under ice-cooling. To the mixture was added diisopropylethylamine (556 ml, 3.19 mol) dropwise at a temperature below 10°C. The mixture was stirred at room temperature overnight. To the resulting solution were added brine and saturated aqueous sodium hydrogen carbonate solution, and the mixture was extracted with ethyl acetate (3.0 L). The aqueous layer was extracted with tetrahydrofuran/ethyl acetate=1/1 (3.0 L) twice.

The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether (1.0 L) and dried in vacuo to give tert-butyl 2-[(5-amino-1-

methyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate (307 g).

IR(KBr) 3440, 3349, 1670, 1631, 1525, 1276, 1163, 1074, 1014, 860, 791 cm^{-1}

5 $^1\text{H-NMR}$ (DMSO- d_6) δ 1.39 (9H, s), 3.44 (3H, s), 3.64 (2H, d, $J=5.9\text{Hz}$), 4.91 (2H, brs), 6.97 (1H, t, $J=5.9\text{Hz}$), 7.15 (1H, s), 9.09 (1H, brs)

Preparation 15

To a solution of tert-butyl 2-[(5-amino-1-methyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate (307 g, 1.14 mol) in N,N-dimethylformamide (1.5 L) was added triphenylmethyl chloride (334 g, 1.2 mol). To the mixture was added triethylamine (318 ml, 2.28 mol) dropwise. The mixture was stirred at room temperature for 1 hour. The reaction mixture was dissolved in ethyl acetate. The solution was washed successively with water, 10% aqueous citric acid solution, water, and brine. The extract was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with acetonitrile (1.5 L) and dried in vacuo to give tert-butyl 2-[[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino]-2-oxoethylcarbamate (468 g).

IR(KBr) 3336, 3280, 1724, 1683, 1599, 1234, 939, 704 cm^{-1}

25 $^1\text{H-NMR}$ (DMSO- d_6) δ 1.38 (9H, s), 2.73 (3H, s), 3.38 (2H, d, $J=5.8\text{Hz}$), 5.58 (1H, s), 6.94 (1H, t, $J=5.8\text{Hz}$), 7.11-7.35 (15H, m), 7.21 (1H, s), 8.31 (1H, s)

ESI-MS: $m/z=512.3$ ($M+H^+$)

30 Example 6

To a solution of benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (489 g) in N,N-dimethylformamide (1.4 L) was added sodium iodide (102 g). After stirring at room temperature for 1 hour, tert-butyl 2-[[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino]-2-oxoethylcarbamate (383 g) was added to the mixture. Stirring was

continued at 37°C for 24 hours. The resulting mixture was poured into water and extracted with ethyl acetate. The organic layer was washed successively with water, 10% aqueous sodium thiosulfate solution, brine and 10% aqueous sodium trifluoroacetate solution, and dried over magnesium sulfate, filtered, and evaporated in vacuo. The residue was dissolved in ethyl acetate (3.5 L), and the solution was dropwise added to diisopropyl ether (36 L). The precipitate was collected by filtration. The filter cake was washed with diisopropyl ether and dried in vacuo.

The obtained solid (700 g) was dissolved in dichloromethane (1.4 L) and to the solution were added anisole (700 ml) and trifluoroacetic acid (2.1 L) successively. After stirring at room temperature for 4 hours, the reaction mixture was poured into diisopropyl ether (30 L). The precipitate was collected by filtration. The obtained solid was washed with diisopropyl ether and dried in vacuo. The crude product was dissolved in water (3.5 L), and the pH of the solution was adjusted to 7.0 with 28% aqueous ammonia solution. The insoluble material was filtered off, and the pH of the filtrate was adjusted to 1 with concentrated hydrochloric acid, and the insoluble material was filtered off again. The filtrate was chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.0 L in vacuo and 2.0M sulfuric acid (102 ml) was added to the concentrate. The mixture was freeze-dried to give the crude product.

The crude product was purified by preparative HPLC (pH 7.0 phosphate buffer and acetonitrile), and the eluate containing a desired product was concentrated to about 6 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 550 ml in vacuo and 2.0M sulfuric acid (54.5 ml)

was added to the concentrate. To the mixture was added dropwise acetonitrile (880 ml) and the mixture was stirred at room temperature overnight. To the mixture was added acetonitrile (200 ml) and the mixture was stirred at room temperature for 2 hours. The resulting white crystals were collected by filtration and washed with 25% aqueous acetonitrile and dried under reduced pressure to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate hydrogen sulfate (72.5 g).
IR(KBr) 1778, 1700, 1653, 1525, 1149, 1111, 617 cm⁻¹
¹H-NMR(D₂O) δ 1.61 (6H, s), 3.22 and 3.45 (2H, ABq, J=17.8Hz), 3.73 (3H, s), 4.03 (2H, s), 5.05 and 5.27 (2H, ABq, J=15.8Hz), 5.25 (1H, d, J=4.8Hz), 5.87 (1H, d, J=4.8Hz), 8.09 (1H, s)
ESI-MS: m/z=638.2 (M+H⁺)

Example 7

A solution of 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (36 g) in water was purified by preparative HPLC utilizing ODS column. The eluate containing a desired product was concentrated to about 1.5 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (6 L) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 800 ml in vacuo and 2M sulfuric acid (17 ml) was added. The resulting solution was lyophilized to give a sulfuric acid salt as an amorphous powder (23.6 g).

The powder was dissolved in water (71 ml) and ethanol (57 ml). After addition of seed crystals (310 mg), which resulted in the precipitation of white solid, the mixture was stirred for 1 hour. A mixture of ethanol (47 ml) and water (37 ml) was added over 30 minutes and ethanol (33 ml) was added over 20 minutes. Then the slurry was stirred for an additional 1.5 hour.

The precipitate was collected by filtration, washed with ethanol/water (60 ml/20 ml) and ethanol (60 ml) and dried to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethyl)ureido]-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate hydrogen sulfate as crystals (17.3 g).

IR(KBr) 3353, 3183, 1778, 1652, 1558, 1403, 1321, 1143, 1118, 997, 619 cm^{-1}

¹H-NMR(D₂O) δ 1.61 (6H, s), 3.10-3.55 (6H, m), 3.71 (3H, s), 5.02 and 5.23 (2H, ABq, J=16.7Hz), 5.25 (1H, d, J=4.9Hz), 5.87 (1H, d, J=4.9Hz), 7.91 (1H, s)

ESI-MS: m/z=667 (M+H⁺)

X-ray powder diffraction analysis (by Rigaku X-ray

Diffraction system MultiFlex)

	2 θ	intensity
	8.0	1286
	12.7	586
	13.8	423
20	16.1	618
	18.9	520
	20.4	748
	21.5	667
	22.4	1058
25	23.3	944
	24.0	618
	25.5	813
	26.7	472
	27.9	537
30	28.5	455
	31.3	390

X-ray: Cu/40 kV/30 mA

Preparation 16

5-Amino-1-ethyl-4-nitrosopyrazole

The title compound was obtained from 5-amino-1-ethylpyrazole in the same manner as in Preparation 3.

¹H-NMR(DMSO-d₆) δ 1.21 (3H, t, J=7.1Hz), 3.93 (2H, q, J=7.1Hz), 7.04 and 8.53 (1H, s), 8.10 and 8.15 (1H, brs)

APCI-MS: $m/z=141(M+H)^+$

Preparation 17

4,5-Diamino-1-ethylpyrazole sulfuric acid salt

The title compound was obtained from 5-amino-1-ethyl-4-nitrosopyrazole in the same manner as in Preparation 4.

1H -NMR(D_2O) δ 1.36 (3H, t, $J=7.3Hz$), 4.10 (2H, q, $J=7.3Hz$), 7.77 (1H, s)

ESI-MS: $m/z=127(M+H)^+$

10 Preparation 18

5-Amino-4-[3-(tert-butoxycarbonylamino)-propionylamino]-1-ethylpyrazole

The title compound was obtained from 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 14.

1H -NMR($DMSO-d_6$) δ 1.24 (3H, t, $J=7.2Hz$), 1.37 (9H, s), 2.35 (2H, t, $J=7.1Hz$), 3.18 (2H, dt, $J=7.1Hz$, 7.1Hz), 3.85 (q, $J=7.2Hz$), 4.88 (2H, brs), 6.75-6.90 (1H, m), 7.17 (1H, s), 9.05 (1H, brs)

20 APCI-MS: $m/z=298(M+H)^+$

Preparation 19

4-[3-(tert-Butoxycarbonylamino)propionylamino]-1-ethyl-5-triphenylmethylaminopyrazole

The title compound was obtained from 5-amino-4-[3-(tert-butoxycarbonylamino)propionylamino]-1-ethylpyrazole in the same manner as in Preparation 15.

1H -NMR($DMSO-d_6$) δ 0.88 (3H, t, $J=7.2Hz$), 1.39 (9H, s), 2.02 (2H, t, $J=7.1Hz$), 2.95-3.20 (4H, m), 5.59 (1H, brs), 6.60-6.75 (1H, m), 7.10-7.35 (16H, m), 8.04 (1H, brs)

30 ESI-MS: $m/z=540(M+H)^+$, 562($M+Na$) $^+$

Example 8

7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionylamino)-2-ethyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-

iodomethyl-3-cephem-4-carboxylate and 4-[3-(tert-butoxycarbonylamino)propionylamino]-1-ethyl-5-triphenylmethylaminopyrazole in the same manner as in Example 1.

- 5 IR(KBr) 3415, 1763, 1658, 1598, 1529, 1402, 1361 cm^{-1}
 $^1\text{H-NMR}(\text{D}_2\text{O})$ δ 1.33 (3H, t, $J=7.2\text{Hz}$), 1.53 (6H, s), 2.89 (2H, t, $J=6.5\text{Hz}$), 3.17 and 3.49 (2H, ABq, $J=17.7\text{Hz}$), 3.34 (2H, t, $J=6.5\text{Hz}$), 4.28 (2H, q, $J=7.2\text{Hz}$), 5.05 and 5.16 (2H, ABq, $J=15.4\text{Hz}$), 5.26 (1H, d, $J=4.8\text{Hz}$), 5.85 (1H, d, $J=4.8\text{Hz}$), 8.03 (1H, s)

Preparation 20

tert-Butyl 2-[(5-amino-1-ethyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate

- 15 The title compound was obtained from 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 14.

- $^1\text{H-NMR}(\text{DMSO}-d_6)$ δ 1.21 (3H, t, $J=7.2\text{Hz}$), 1.39 (9H, s), 3.64 (2H, d, $J=6.0\text{Hz}$), 3.86 (2H, d, $J=7.2\text{Hz}$), 4.88 (2H, brs), 6.90-7.00 (1H, m), 7.17 (1H, s), 9.06 (1H, brs)
20 ESI-MS: $m/z=284(\text{M}+\text{H})^+$, $306(\text{M}+\text{Na})^+$

Preparation 21

tert-Butyl 2-[[1-ethyl-5-(tritylamino)-1H-pyrazol-4-yl]amino]-2-oxoethylcarbamate

- 25 The title compound was obtained from tert-butyl 2-[(5-amino-1-ethyl-1H-pyrazol-4-yl)amino]-2-oxoethylcarbamate in the same manner as in Preparation 15.

- $^1\text{H-NMR}(\text{DMSO}-d_6)$ δ 0.88 (3H, t, $J=7.2\text{Hz}$), 1.38 (9H, s), 3.16 (2H, q, $J=7.2\text{Hz}$), 3.31 (2H, d), 5.59 (1H, brs), 6.80-6.95 (1H, m), 7.10-7.40 (16H, m), 8.03 (1H, brs)
30 ESI-MS: $m/z=526(\text{M}+\text{H})^+$, $548(\text{M}+\text{Na})^+$

Example 9

- 7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(aminoacetyl)amino-2-ethyl-1-pyrazolio]methyl-3-cephem-4-carboxylate
35

The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-

butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and tert-butyl 2-([1-ethyl-5-(tritylamino)-1H-pyrazol-4-yl]amino)-2-oxoethylcarbamate in the same manner as in Example 1.

5 IR(KBr) 3444, 1761, 1635, 1626, 1446, 1406 cm^{-1}

$^1\text{H-NMR}(\text{D}_2\text{O})$ δ 1.33 (3H, t, $J=7.2\text{Hz}$), 1.53 (6H, s), 2.89 (2H, t, $J=6.5\text{Hz}$), 3.17 and 3.49 (2H, ABq, $J=17.7\text{Hz}$), 4.00 (2H, s), 4.28 (2H, q, $J=7.2\text{Hz}$), 5.06 and 5.17 (2H, ABq, $J=15.4\text{Hz}$), 5.27 (1H, d, $J=4.8\text{Hz}$), 5.85 (1H, d, $J=4.8\text{Hz}$), 8.07 (1H, s)

Preparation 22

5-Amino-4-[2',3'-bis(tert-butoxycarbonyl)-guanidino]-1-ethylpyrazole

15 The title compound was obtained from 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethylsulfonyl)guanidine and 4,5-diamino-1-ethylpyrazole sulfuric acid salt in the same manner as in Preparation 12.

$^1\text{H-NMR}(\text{DMSO}-d_6)$ δ 1.22 (3H, t, $J=7.1\text{Hz}$), 1.37 (9H, s), 1.50 (9H, s), 3.88 (2H, d, $J=7.1\text{Hz}$), 5.12 (2H, brs), 7.14 (1H, s), 9.16 (1H, brs), 11.51 (1H, brs)

20 ESI-MS: $m/z=369(\text{M}+\text{H})^+$

Preparation 23

4-[2',3'-Bis(tert-butoxycarbonyl)guanidino]-1-ethyl-5-triphenylmethylaminopyrazole

25 The title compound was obtained from 5-amino-4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-ethylpyrazole in the same manner as in Preparation 15.

$^1\text{H-NMR}(\text{DMSO}-d_6)$ δ 0.86 (3H, t, $J=7.1\text{Hz}$), 1.38 (9H, s), 1.49 (9H, s), 5.85 (1H, brs), 7.10-7.30 (16H, m), 8.80 (1H, brs), 11.14 (1H, brs)

30 ESI-MS: $m/z=611(\text{M}+\text{H})^+$, 633($\text{M}+\text{Na}$) $^+$

Example 10

7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-2-ethyl-4-guanidino-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-t rt-

butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-ethyl-5-triphenylmethylaminopyrazole in the same manner as in

5 Example 1.

IR(KBr) 3437, 1760, 1658, 1625, 1406, 1065 cm^{-1}

$^1\text{H-NMR}(\text{D}_2\text{O})$ δ 1.35 (3H, t, $J=7.3\text{Hz}$), 1.53 (6H, s), 3.26 and 3.61 (2H, ABq, $J=17.8\text{Hz}$), 4.29 (2H, q, $J=7.3\text{Hz}$), 5.06 and 5.17 (2H, ABq, $J=15.7\text{Hz}$), 5.29 (1H, d, $J=4.8\text{Hz}$), 5.85 (1H, d, $J=4.8\text{Hz}$), 8.06 (1H, s)

10

Example 11

To a suspension of benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (500 g, 611 mmol) in N,N-dimethylformamide (2.5 L) was added 4-[2',3'-bis(tert-butoxycarbonyl)guanidino]-1-methyl-5-triphenylmethylaminopyrazole (419 g) and the mixture was stirred at room temperature for 16 hours. The reaction mixture was added to a mixture of ethyl acetate and water. The organic layer was washed with water, brine and 10% aqueous sodium trifluoroacetate solution and then dried over magnesium sulfate. The magnesium sulfate was filtered off, and the filtrate was evaporated to 3.3 kg under reduced pressure. The concentrate was poured into diisopropyl ether (33 L), and the resulting precipitate was collected by filtration and dried in vacuo.

To a solution of the resulting solid in methylene chloride (1500 ml) were added anisole (500 ml) and trifluoroacetic acid (1500 ml). The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether. The resulting precipitate was collected by filtration and dried in vacuo. The crude product was dissolved in water (3.5 L), and the pH of the solution was adjusted to 7.0 with 28% aqueous ammonia solution. The insoluble material was filtered off, and the pH of the filtrate was adjusted to 1 with concentrated hydrochloric acid, and the insoluble

material was filtered off, again. The filtrate was chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.0 L in vacuo. To the concentrate was added 2.0M sulfuric acid (150 ml) and the mixture was freeze-dried to give the crude product. The crude product was purified with preparative HPLC utilizing ODS column (pH 7.0 phosphate buffer and acetonitrile). The eluate containing a desired product was concentrated to about 6 L in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 1.5 L in vacuo. To the concentrate was added 2.0M sulfuric acid (60 ml) and the mixture was freeze-dried to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-guanidino-2-methyl-1-pyrazolio)methyl-3-cephem-4-carboxylate hydrogen sulfate (48.5 g, yield 11%).

IR(KBr) 1776, 1714, 1677, 1651, 1402, 1112 cm⁻¹

¹H-NMR(D₂O) δ 1.61 (6H, s), 3.28 and 3.58 (2H, ABq, J=17.8Hz), 3.74 (3H, s), 5.15 and 5.23 (2H, ABq, J=15.7Hz), 5.27 (1H, d, J=4.8Hz), 5.88 (1H, d, J=4.8Hz), 8.07 (1H, s)

ESI-MS: m/z=623.2 (M+H⁺)

Preparation 24

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (2.4 g, 10 mmol) in methylene chloride (40 ml) were added N-ethyldiisopropylamine (2.1 ml, 12 mmol) and N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (2.3 g, 8 mmol) under ice-cooling, and the mixture was stirred at room temperature for 6 hours. To the reaction mixture were added brine (40 ml) and saturated aqueous sodium hydrogen carbonate solution (20 ml), and the mixture was extracted with a mixture of ethyl acetate and 2-propanol (3:1, 60 ml). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in

vacuo. The residue was triturated with diethyl ether to give 5-amino-4-[3-(tert-butoxycarbonylamino)propionyl]amino-1-(2-hydroxyethyl)pyrazole (1.65 g) as a solid.

- 5 $^1\text{H-NMR}$ (DMSO-d_6) δ 1.38 (9H, s), 2.35 (2H, t, $J=7.3\text{Hz}$), 3.16-3.20 (2H, m), 3.62-3.65 (2H, m), 3.90 (2H, t, $J=6.0\text{Hz}$), 4.85 (2H, brs), 4.92 (1H, t, $J=5.0\text{Hz}$), 6.84 (1H, t, $J=5.5\text{Hz}$), 7.20 (1H, s), 9.09 (1H, brs).

Example 12

- 10 7β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(3-aminopropionamido)-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl

- 15 7β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[3-(tert-butoxycarbonylamino)propionyl]amino-1-(2-hydroxyethyl)pyrazole in the same manner as in

- 20 Example 1 as an amorphous solid.

- $^1\text{H-NMR}$ (D_2O) δ 1.51 (6H, s), 2.88 (2H, t, $J=6.4\text{Hz}$), 3.15 (1H, d, $J=17.9\text{Hz}$), 3.48 (1H, d, $J=17.9\text{Hz}$), 3.32 (2H, t, $J=6.4\text{Hz}$), 3.88 (2H, t, $J=4.8\text{Hz}$), 4.39 (1H, dt, $J=16.5\text{Hz}$, 4.8Hz), 4.42 (1H, dt, $J=16.5\text{Hz}$, 4.8Hz), 5.06 (1H, d, $J=15.1\text{Hz}$), 5.11 (1H, d, $J=15.1\text{Hz}$), 5.25 (1H, d, $J=5.0\text{Hz}$), 5.83 (1H, d, $J=5.0\text{Hz}$), 8.05 (1H, s).
- 25

Preparation 25

- To a solution of 4-formyl-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (1.51 g, 10 mmol) in sulfuric acid (7.5 ml) was added potassium nitrate (111 g, 1.1 mol) under ice-cooling. The mixture was stirred at room temperature for 17 hours. The reaction mixture was added to ice (100 g). The crystalline residue was collected by filtration and dried in vacuo to give 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (0.63 g) as a solid.
- 30
- 35

- $^1\text{H-NMR}$ (DMSO-d_6) δ 2.00-2.05 (2H, m), 3.30-3.36 (2H, m), 3.99 (2H, t, $J=6.0\text{Hz}$), 7.85 (1H, s), 7.89 (1H, s)

Preparation 26

A solution of 3-nitro-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (1.68 g, 10 mmol) in a mixture of sulfuric acid (0.6 ml), acetic acid (100 ml) and water (10 ml) was treated with 10% palladium on carbon (0.5 g) under a hydrogen atmosphere at room temperature for 6 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with ethanol and dried in vacuo to give 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.3 g) as a solid.
¹H-NMR(DMSO-d₆) δ 1.97-2.01 (2H, m), 3.22 (2H, t, J=5.0Hz), 3.98 (2H, t, J=6.0Hz), 7.22 (1H, s)

Preparation 27

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.96 g, 10 mmol) and N-ethyldiisopropylamine (3.88 g, 30 mmol) in methylene chloride (70 ml) was added 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)-guanidine (3.91 g, 10 mmol). The mixture was stirred at room temperature for 150 minutes. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 2% methanol/chloroform to give 3-[2,3-bis(tert-butoxycarbonyl)guanidino]-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (3.4 g) as a solid.
¹H-NMR(CDCl₃) δ 1.48 (9H, s), 1.52 (9H, s), 2.12-2.14 (2H, m), 3.33-3.37 (2H, m), 4.08 (2H, t, J=6.0Hz), 6.17 (1H, brs), 7.16 (1H, s), 9.87 (1H, brs), 11.39 (1H, brs)

Example 13

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.2 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide

(181 mg, 1.2 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture were added 3-[2,3-bis(tert-butoxycarbonyl)guanidino]-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (571 mg, 1.5 mmol) and methylene chloride (2.0 ml). The whole mixture was stirred at room temperature for 7 hours. To the reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (150 ml), and the resulting precipitate was collected by filtration and dried in vacuo.

To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml) and the mixture was stirred at room temperature for 4 hours. The reaction mixture was poured into diisopropyl ether (150 ml) and the resulting precipitate was collected by filtration and dried in vacuo to give a crude product (570 mg), which was purified by preparative HPLC utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-guanidino-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate (51 mg) as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 2.05-2.25 (2H, m), 3.26 (1H, d, J=17.4Hz), 3.56 (1H, d, J=17.4Hz), 3.30-3.45 (2H, m), 4.15 (2H, t, J=6.0Hz), 4.93 (1H, d, J=15.6Hz), 5.15 (1H, d, J=15.6Hz), 5.25 (1H, d, J=4.8Hz),

5.84 (1H, d, J=4.8Hz), 7.99 (1H, s)

Preparation 28

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (4.4 g, 20 mmol), 4-(dimethylamino)pyridine (244 mg, 2 mmol) and triethylamine (8.10 g, 80 mmol) in chloroform (45 ml) was added 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)guanidine (10.18 g, 26 mmol). The mixture was stirred at room temperature for 2 hours. The reaction mixture was washed successively with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was triturated with diisopropyl ether to give 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (4.6 g) as a solid. ¹H-NMR(CDCl₃) δ 1.49 (9H, s), 1.52 (9H, s), 3.97-4.01 (2H, m), 4.21 (2H, t, J=7.8Hz), 5.30 (1H, brs), 7.19 (1H, s), 9.86 (1H, brs), 11.32 (1H, brs)

Example 14

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-guanidino-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole in the same manner as in Example 13 as an amorphous solid.

¹H-NMR(D₂O) δ 1.51 (3H, s), 1.52 (3H, s), 3.35 (1H, d, J=17.9Hz), 3.61 (1H, d, J=17.9Hz), 4.19 (2H, t, J=8.7Hz), 4.37 (1H, q, J=8.7Hz), 4.47 (1H, q, J=8.7Hz), 5.00 (1H, d, J=15.1Hz), 5.04 (1H, d, J=15.1Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.13 (1H, s)

Preparation 29

To a solution of 5-amino-1-(2-hydroxyethyl)pyrazole (6.35 g, 50 mmol) in a mixed solvent of ethanol (25 ml) and concentrated hydrochloric acid (0.035 ml) was added dropwise isoamyl nitrite (7.03 g, 60 mmol). The mixture was stirred at room temperature for 17 hours. The crystalline residue was collected by filtration and dried in vacuo to give 5-amino-1-(2-hydroxyethyl)-4-nitrosopyrazole (4.0 g) as a solid.

¹H-NMR(DMSO-d₆) δ 3.68 (2H, t, J=5.5Hz), 3.94 (2H, t, J=5.5Hz), 4.89 (1H, br), 8.06(2H, br); 8.53 (1H, s)

Preparation 30

A solution of 5-amino-1-(2-hydroxyethyl)-4-nitrosopyrazole (97 g, 629 mmol) in a mixed solvent of sulfuric acid (34 ml) and water (2000 ml) was treated with 10% palladium on carbon (10 g) under a hydrogen atmosphere at room temperature for 4 days. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was triturated with methanol and dried in vacuo to give 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (90.2 g) as a solid.

¹H-NMR(DMSO-d₆) δ 3.66 (2H, t, J=5.5Hz), 3.95 (2H, t, J=5.5Hz), 7.25 (1H, s)

Preparation 31

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (50.0 g, 208 mmol) in chloroform (500 ml) were added 4-(dimethylamino)pyridine (2.54 g, 20.8 mmol), triethylamine (116 ml, 833 mmol) and 1,3-bis(tert-butoxycarbonyl)-2-(trifluoromethanesulfonyl)guanidine (106 g, 271 mmol). The mixture was stirred under reflux for 2 hours. After cooling on an ice bath, the reaction mixture was washed successively with water, 4% aqueous citric acid solution, water and aqueous sodium hydrogen carbonate solution. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was triturated with a mixed solvent of ethyl

acetate (50 ml) and diethyl ether (200 ml) to give 5-amino-4-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-(2-hydroxyethyl)pyrazole (50 g) as a solid.

¹H-NMR(CDCl₃) δ 1.47 (9H, s), 1.53 (9H, s), 3.28 (1H, br),
5 4.02-4.05 (4H, m), 4.65 (2H, br), 7.22 (1H, s), 9.85 (1H, br), 11.55 (1H, br)

Example 15

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-guanidino-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-cephem-4-carboxylate
10

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-(2-hydroxyethyl)pyrazole in the same manner as in Example 13 as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 3.21 (1H, d, J=17.9Hz), 3.59
20 (1H, d, J=17.9Hz), 3.90 (2H, t, J=4.8Hz), 4.35-4.50 (2H, m), 5.07 (1H, d, J=14.9Hz), 5.11 (1H, d, J=14.9Hz), 5.28 (1H, d, J=5.0Hz), 5.84 (1H, d, J=5.0Hz), 8.09 (1H, s)

Preparation 32

To a solution of 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.83 g, 5 mmol) in pyridine (10 ml) was added triphenylmethyl chloride (1.67 g, 6 mmol). The mixture was stirred at 50°C for 5 hours. After cooling, chloroform (50 ml) was added to the reaction mixture, and the mixture was washed successively with 10% aqueous citric acid solution, brine, and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 2% methanol/chloroform to give 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-triphenylmethyl-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.57 g) as a solid.
35

¹H-NMR(CDCl₃) δ 1.47 (9H, s), 1.48 (9H, s), 3.50 (2H, t, J=7.8Hz), 3.92 (2H, t, J=7.8Hz), 7.07-7.26 (10H, m), 7.53-7.54 (6H, m), 8.34 (1H, brs), 11.12 (1H, brs)

Example 16

5 To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (819 mg, 1.0 mmol) in N,N-dimethylformamide (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 10 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-[2,3-bis(tert-butoxycarbonyl)guanidino]-1-triphenylmethyl-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (730 mg, 1.2 mmol). The whole mixture was stirred at 15 room temperature for 6 hours. To the resulting reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution, 10% aqueous sodium 20 thiosulfate solution and brine, dried over sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (120 ml), and the resulting precipitate was collected by filtration and dried in 25 vacuo.

 To a solution of the resulting solid in methylene chloride (2.0 ml) were added anisole (0.67 ml) and trifluoroacetic acid (1.34 ml) and the mixture was stirred at room temperature for 4 hours. The reaction 30 mixture was poured into diisopropyl ether (120 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (430 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate 35 containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical

Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-guanidino-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (20.4 mg) as an amorphous solid.

¹H-NMR(D₂O) δ 1.51 (3H, s), 1.52 (3H, s), 3.35 (1H, d, J=17.9Hz), 3.61 (1H, d, J=17.9Hz), 4.19 (2H, t, J=8.7Hz), 4.37 (1H, q, J=8.7Hz), 4.47 (1H, q, J=8.7Hz), 5.00 (1H, d, J=15.1Hz), 5.04 (1H, d, J=15.1Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.13 (1H, s)

Preparation 33

To a suspension of 1,1'-carbonyldiimidazole (1.94 g, 12 mmol) in methylene chloride (20 ml) was added tert-butyl N-(3-aminopropyl)carbamate (2.30 g, 13.2 mmol), and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyl-diisopropylamine (2.56 g, 20 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (2.10 g, 10 mmol), and the mixture was stirred at 30°C for 3 days. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 6% methanol/chloroform to give 5-amino-4-(3-{3-[(tert-butoxycarbonyl)amino]propyl}ureido)-1-methylpyrazole (1.75 g) as a solid.

¹H-NMR(DMSO-d₆) δ 1.37 (9H, s), 1.43-1.49 (2H, m), 2.89-2.93 (2H, m), 2.98-3.01 (2H, m), 3.50 (3H, s), 4.79 (2H, br), 5.85 (1H, br), 6.77 (1H, br), 6.96 (1H, s), 7.12 (1H, br)

Example 17

To a solution of benzhydryl 7 β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.21 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide (199 mg, 1.33 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction

mixture was added 5-amino-4-(3-{3-[(tert-butoxycarbonyl)amino]propyl}ureido)-1-methylpyrazole (415 mg, 1.33 mmol) and the whole mixture was stirred at 32°C for 24 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (100 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (3.6 ml) were added anisole (1.2 ml) and trifluoroacetic acid (2.4 ml). The resulting solution was stirred at room temperature for 4 hours and poured into diisopropyl ether (100 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (939 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(2Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-[3-(3-aminopropyl)ureido]-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate (53 mg) as an amorphous solid.

¹H-NMR(D₂O) δ: 1.52 (3H, s), 1.53 (3H, s), 1.85-1.88 (2H, m), 3.03 (2H, t, J=8Hz), 3.22 (2H, t, J=18Hz), 3.26 (2H, t, J=7Hz), 3.49 (1H, d, J=18Hz), 3.72 (3H, s), 4.96 (1H, d, J=15Hz), 5.16 (1H, d, J=15Hz), 5.25 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 7.88 (1H, s)

Preparation 34

To a suspension of 1,1'-carbonyldiimidazole (973

mg, 6 mmol) in methylene chloride (10 ml) was added
tert-butyl N-(2-aminoethyl)carbamate (1.11 g, 6.9 mmol)
under ice-cooling, and the mixture was stirred at room
temperature for 2 hours. To the reaction mixture were
5 added N-ethyl-diisopropylamine (1.28 g, 10 mmol) and 3-
amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine
sulfuric acid salt (1.18 g, 5 mmol), and the mixture was
stirred at 50°C for 6 hours. The reaction mixture was
washed with brine. The organic layer was dried over
10 anhydrous magnesium sulfate, filtered, and concentrated
in vacuo. The residue was purified by column
chromatography on silica gel eluting with 5%
methanol/chloroform to give 3-(3-(2-((tert-
butoxycarbonyl)amino)ethyl)ureido)-4,5,6,7-
15 tetrahydropyrazolo[1,5-a]pyrimidine (150 mg) as a solid.
¹H-NMR(CDCl₃) δ 1.43 (9H, s), 2.11-2.16 (2H, m), 3.22-
3.35 (6H, m), 4.09 (2H, t, J=7Hz), 4.69 (1H, br), 5.14
(2H, br), 5.69 (1H, br), 7.17 (1H, s)

Example 18

20 7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-
carboxy-1-methylethoxyimino)acetamido]-3-(3-(3-(2-
aminoethyl)ureido)-4,5,6,7-tetrahydro-1-pyrazolo[1,5-
a]pyrimidinio)methyl-3-cephem-4-carboxylate.

The title compound was obtained from benzhydryl
25 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-
3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-
acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-
(3-(2-((tert-butoxycarbonyl)amino)ethyl)ureido)-4,5,6,7-
tetrahydropyrazolo[1,5-a]pyrimidine in the same manner
30 as in Example 17 as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 2.09-2.21 (2H,
m), 3.13 (2H, t, J=6Hz), 3.24 (1H, d, J=18Hz), 3.35-3.52
(5H, m), 4.12-4.15 (2H, m), 4.88 (1H, d, J=16Hz), 5.13
(1H, d, J=16Hz), 5.25 (1H, d, J=5Hz), 5.85 (1H, d,
35 J=5Hz), 7.83 (1H, s)

Preparation 35

To a suspension of 1,1'-carbonyldiimidazole (973
mg, 6 mmol) in methylene chloride (10 ml) was added O-

[2-(tert-butoxycarbonylamino)ethyl]hydroxylamine (1.11 g, 6.3 mmol) under ice-cooling, and the mixture was stirred at room temperature for 2 hours. To the reaction mixture were added N-ethyldiisopropylamine (1.28 g, 10 mmol) and 4,5-diamino-1-methylpyrazole sulfuric acid salt (1.05 g, 5 mmol), and the mixture was stirred under reflux for 4 hours. The reaction mixture was washed with brine. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 10% methanol/chloroform to give 5-amino-4-(3-(2-((tert-butoxycarbonyl)amino)ethoxy)-ureido)-1-methylpyrazole (255 mg) as a solid.

¹H-NMR(DMSO-d₆) δ 1.38 (9H, s), 3.19-3.20 (2H, m), 3.51 (3H, s), 3.72 (2H, t, J=6Hz), 4.86 (2H, br), 6.95 (1H, br), 7.06 (1H, s), 8.02 (1H, brs), 9.15 (1H, brs)

Example 19

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[3-(2-aminoethoxy)ureido]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-(3-(2-((tert-butoxycarbonyl)amino)ethoxy)-ureido)-1-methylpyrazole in the same manner as in Example 17 as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 3.21 (1H, d, J=18Hz), 3.33 (2H, t, J=5Hz), 3.47 (1H, d, J=18Hz), 3.74 (3H, s), 4.17 (2H, t, J=5Hz), 4.99 (1H, d, J=15Hz), 5.17 (1H, d, J=15Hz), 5.26 (1H, d, J=5Hz), 5.86 (1H, d, J=5Hz), 7.93 (1H, s)

Preparation 36

To a suspension of 1,1'-carbonyldiimidazole (1.95 g, 12 mmol) in methylene chloride (20 ml) was added tert-butyl N-(2-aminoethyl)carbamate (1.92 g, 12 mmol) under ice-cooling, and the mixture was stirred at room

temperature for 2 hours. To the reaction mixture were added N-ethyldiisopropylamine (2.59 g, 20 mmol) and 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (2.22 g, 10 mmol), and the mixture was stirred
5 at room temperature for 16 hours. To the reaction mixture were added trityl chloride (9.0 g, 32 mmol) and triethylamine (3.0 g, 30 mmol). The mixture was stirred at room temperature for 24 hours. The reaction mixture was washed with 10% aqueous citric acid solution, brine
10 and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 3% methanol/chloroform to give 7-(3-{2-
15 [(tert-butoxycarbonyl)amino]ethyl}ureido)-2,3-dihydro-1-tritylimidazo[1,2-b]pyrazole (800 mg) as a solid.

¹H-NMR(CDCl₃) δ 1.43 (9H, s), 3.19 (4H, br), 3.69 (1H, brs), 3.78-3.85 (4H, m), 4.51 (1H, br), 5.07 (1H, br), 7.20 (1H, s), 7.26-7.34 (9H, m), 7.46-7.47 (6H, m)

20 Example 20

To a solution of benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate (820 mg, 1.0 mmol) in N,N-dimethylformamide
25 (2.4 ml) was added N-trimethylsilylacetamide (656 mg, 5.0 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-(3-{2-[(tert-butoxycarbonyl)amino]ethyl}ureido)-2,3-dihydro-1-tritylimidazo[1,2-b]pyrazole (700 mg, 1.2
30 mmol) and the whole mixture was stirred at room temperature for 6 hours. To the resulting reaction mixture were added ethyl acetate (50 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium
35 trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (120 ml), and the

resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml). The resulting
5 solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (120 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (830 mg), which was purified by preparative high-performance liquid
10 chromatography (HPLC) utilizing ODS column. The eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical
15 Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{7-[3-(2-aminoethyl)ureido]-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)}methyl-3-cephem-4-carboxylate (28.5 mg) as
20 an amorphous solid.

¹H-NMR(D₂O) δ 1.53 (3H, s), 1.54 (3H, s), 3.14 (2H, t, J=6Hz), 3.29 (1H, d, J=18Hz), 3.49 (2H, t, J=6Hz), 3.57 (1H, d, J=18Hz), 4.16 (2H, t, J=9Hz), 4.31-4.45 (2H, m),
25 4.94 (1H, d, J=15Hz), 5.02 (1H, d, J=15Hz), 5.27 (1H, d, J=5Hz), 5.85 (1H, d, J=5Hz), 7.95 (1H, s)

Preparation 37

To a suspension of 1,1'-carbonyldiimidazole (2.0 g, 12.3 mmol) in dehydrated chloroform (30 ml) was added a
30 solution of tert-butyl N-(2-hydroxyethyl)carbamate (1.92 g, 12 mmol) in dehydrated chloroform (10 ml) under ice-cooling, and the mixture was stirred at room temperature for 1 hour. To the reaction mixture were added N-ethyl-diisopropylamine (2.2 ml, 12.3 mmol) and 4,5-
35 diamino-1-methylpyrazole sulfuric acid salt (2.58 g, 12.3 mmol), and the mixture was stirred at room temperature for 17.5 hours. To the reaction mixture were added trityl chloride (3.42 g, 12.3 mmol) and

triethylamine (1.25 g, 12.3 mmol). The mixture was stirred at room temperature for 2 hours. The reaction mixture was washed with 10% aqueous citric acid solution, brine and saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 4-[[2-(tert-butoxycarbonylamino)ethoxycarbonyl]amino]-5-(tritylamino)-1-methylpyrazole (1.91 g) as a solid.

¹H-NMR(CDCl₃) δ 1.46 (9H, s), 2.89 (3H, s), 3.30-3.36 (2H, m), 4.03-4.07 (2H, m), 4.37 (1H, brs), 4.75 (1H, br), 5.42 (1H, br), 7.17-7.30 (16H, m)

Example 21

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-[(2-aminoethoxycarbonyl)amino]-2-methyl-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl

7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and 4-[[2-(tert-butoxycarbonylamino)ethoxycarbonyl]amino]-5-(tritylamino)-1-methylpyrazole in the same manner as in Example 20 as an amorphous solid.

¹H-NMR(D₂O) δ 1.53 (3H, s), 1.54 (3H, s), 3.18 (1H, d, J=18Hz), 3.30-3.38 (2H, m), 3.43 (1H, d, J=18Hz), 3.71 (3H, s), 4.37-4.40 (2H, m), 4.97 (1H, d, J=15Hz), 5.18 (1H, d, J=15Hz), 5.24 (1H, d, J=5Hz), 5.83 (1H, d, J=5Hz), 7.95 (1H, s)

Preparation 38

To a solution of 7-amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole sulfuric acid salt (1.42 g, 6.4 mmol) and N-ethyldiisopropylamine (2.73 g, 21 mmol) in methylene chloride (50 ml) was added N-[2-(tert-butoxycarbonylamino)acetoxy]succinimide (1.90 g, 7.0 mmol). The mixture was stirred at room temperature for 22 hours. The reaction mixture was washed with

saturated aqueous sodium hydrogen carbonate solution and the organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 7-[2-(tert-butoxycarbonylamino)acetyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (1.07 g) as a solid.

¹H-NMR(CDCl₃) δ 1.47 (9H, s), 3.89 (2H, d, J=5.5Hz), 3.97 (2H, dt, J=2.7Hz, 7.3Hz), 4.18 (2H, t, J=7.3Hz), 4.55 (1H, br), 5.22 (1H, br), 7.16 (1H, s), 7.95 (1H, br)

Example 22

To a solution of benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (1.0 g, 1.2 mmol) in N,N-dimethylformamide (2.0 ml) was added sodium iodide (181 mg, 1.2 mmol), and the mixture was stirred at room temperature for 30 minutes. To the reaction mixture was added 7-[2-(tert-butoxycarbonylamino)acetyl]amino-2,3-dihydro-1H-imidazo[1,2-b]pyrazole (421 mg, 1.5 mmol). The whole mixture was stirred at 30°C for 3 hours. To the resulting reaction mixture were added ethyl acetate (100 ml) and water (50 ml). The aqueous layer was separated, and the organic layer was washed with 10% aqueous sodium trifluoroacetate solution and brine, dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated to about 5 ml in vacuo. The concentrate was poured into diisopropyl ether (150 ml), and the resulting precipitate was collected by filtration and dried in vacuo. To a solution of the resulting solid in methylene chloride (3.0 ml) were added anisole (1.0 ml) and trifluoroacetic acid (2.0 ml). The resulting solution was stirred at room temperature for 4 hours, and poured into diisopropyl ether (150 ml). The resulting precipitate was collected by filtration and dried in vacuo to give a crude product (830 mg), which was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The

eluate containing a desired product was concentrated to about 30 ml in vacuo. The concentrate was adjusted to about pH 3 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (Mitsubishi Chemical Corporation) eluting with 30% aqueous 2-propanol. The eluate was concentrated to about 30 ml in vacuo and lyophilized to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[7-(2-aminoacetamido)-2,3-dihydro-5-(1H-imidazo[1,2-b]pyrazolio)]methyl-3-cephem-4-carboxylate (20.8 mg) as an amorphous solid.

¹H-NMR(D₂O) δ 1.51 (3H, s), 1.52 (3H, s), 3.26 (2H, d, J=18Hz), 3.54 (2H, d, J=18Hz), 3.97 (2H, s), 4.16 (2H, t, J=9Hz), 4.35 (1H, q, J=9Hz), 4.44 (1H, q, J=9Hz), 4.97 (2H, d, J=15Hz), 5.04 (2H, d, J=15Hz), 5.25 (1H, d, J=4Hz), 5.84 (1H, d, J=4Hz), 8.10 (1H, s)

Preparation 39

To a suspension of 4,5-diamino-1-(2-hydroxyethyl)pyrazole sulfuric acid salt (1.20 g, 5 mmol) and N-[2-(tert-butoxycarbonylamino)acetoxy]-succinimide (1.35 g, 5 mmol) in methylene chloride (20 ml) was added N-ethyldiisopropylamine (2.1 ml, 12 mmol) under ice-cooling, and the mixture was stirred at room temperature for 17 hours. The reaction mixture was washed with water (40 ml), saturated aqueous sodium hydrogen carbonate solution (40 ml) and brine (40 ml). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The oily residue was purified by column chromatography on silica gel eluting with 10% methanol/chloroform to give 5-amino-4-[2-(tert-butoxycarbonylamino)acetyl]amino-1-(2-hydroxyethyl)pyrazole (1.20 g) as a solid.

¹H-NMR(CDCl₃) δ 1.46 (9H, s), 3.89-3.90 (4H, m), 4.00-4.04 (2H, m), 4.26 (2H, br), 5.51 (1H, br), 7.17 (1H, s), 8.06 (1H, br)

Example 23

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-amino-4-(2-

aminoacetamido)-2-(2-hydroxyethyl)-1-pyrazolio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 5-amino-4-[2-(tert-butoxycarbonylamino)acetyl]amino-1-(2-hydroxyethyl)pyrazole in the same manner as in Example 22 as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (6H, s), 3.15 (2H, d, J=18Hz), 3.48 (2H, d, J=18Hz), 3.88 (1H, dt, J=16Hz), 4.02 (2H, s), 4.42 (1H, dt, J=16.5Hz), 5.07 (2H, d, J=15Hz), 5.13 (2H, d, J=15Hz), 5.27 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 8.09 (1H, s)

15 Preparation 40

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (2.96 g, 10 mmol) and N-ethyldiisopropylamine (2.59 g, 20 mmol) in methylene chloride (70 ml) was added N-[2-(tert-butoxycarbonylamino)acetoxy]succinimide (2.72 g, 10 mmol). The mixture was stirred at room temperature for 14 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 6% methanol/chloroform to give 3-[2-(tert-butoxycarbonylamino)acetyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (2.4 g) as a solid.

¹H-NMR(CDCl₃) δ 1.46 (9H, s), 2.08-2.12 (2H, m), 3.29-3.32 (2H, m), 3.90 (2H, br), 4.07 (2H, t, J=6.0Hz), 5.00 (1H, br), 5.38 (1H, br), 7.12 (1H, s), 8.11 (1H, br).

Example 24

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-(2-aminoacetamido)-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl

7 β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-[2-(tert-butoxycarbonylamino)acetyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine in the same manner as in Example 22 as an amorphous solid.

¹H-NMR(D₂O) δ 1.52 (3H, s), 1.53 (3H, s), 2.05-2.25 (2H, m), 3.21 (2H, d, J=18Hz), 3.45 (2H, d, J=18Hz), 3.30-3.45 (2H, m), 4.00 (2H, s), 4.10-4.25 (2H, m), 4.92 (2H, d, J=15Hz), 5.17 (2H, d, J=15Hz), 5.24 (1H, d, J=5Hz), 5.84 (1H, d, J=5Hz), 7.97 (1H, s)

Preparation 41

To a solution of 3-amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine sulfuric acid salt (4.44 g, 15 mmol) and N-ethyldiisopropylamine (3.88 g, 30 mmol) in methylene chloride (100 ml) was added N-[3-(tert-butoxycarbonylamino)propionyloxy]succinimide (4.29 g, 15 mmol). The mixture was stirred at room temperature for 6 hours. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 5% methanol/chloroform to give 3-[3-(tert-butoxycarbonylamino)propionyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine (3.67 g) as an oil.

¹H-NMR(CDCl₃) δ 1.43 (9H, s), 2.08-2.13 (2H, m), 2.52 (2H, t, J=6.0Hz), 3.32 (2H, t, J=5.0Hz), 3.43-3.46 (2H, m), 4.07 (2H, t, J=6.0Hz), 5.12 (1H, br), 5.23 (1H, br), 7.13 (1H, s), 7.97 (1H, br)

Example 25

7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-[3-(3-aminopropionamido)-4,5,6,7-tetrahydro-1-pyrazolo[1,5-a]pyrimidinio]methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-tert-butoxycarbonylamino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)-

acetamido]-3-chloromethyl-3-cephem-4-carboxylate and 3-[3-(tert-butoxycarbonylamino)propionyl]amino-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrimidine in the same manner as in Example 22 as an amorphous solid.

- 5 $^1\text{H-NMR}(\text{D}_2\text{O})$ δ 1.51 (3H, s), 1.52 (3H, s), 2.05-2.25 (2H, m), 2.85 (2H, t, $J=7\text{Hz}$), 3.20 (2H, d, $J=18\text{Hz}$), 3.44 (2H, d, $J=18\text{Hz}$), 3.30-3.45 (2H, m), 3.31 (2H, t, $J=7\text{Hz}$), 4.05-4.20 (2H, m), 4.91 (2H, d, $J=16\text{Hz}$), 5.16 (2H, d, $J=16\text{Hz}$), 5.23 (1H, d, $J=5\text{Hz}$), 5.84 (1H, d, $J=5\text{Hz}$), 7.92
10 (1H, s)

Preparation 42

- To a solution of 5-amino-1-methylpyrazole (100 g) in water (700 ml) were added concentrated hydrochloric acid (86 ml) and sodium nitrite (63.9 g) in water (200
15 ml) at a temperature below 10°C . The reaction mixture was stirred at 5°C for 30 minutes. The precipitated solid was collected by filtration and dried to give 5-amino-1-methyl-4-nitrosopyrazole (117 g).

- 20 $^1\text{H-NMR}(\text{DMSO}-d_6)$ δ 3.52 and 3.59 (3H, s), 7.22 and 8.51 (1H, s), 8.17 and 8.51 (1H, brs)

Preparation 43

- To a suspension of 5-amino-1-methyl-4-nitrosopyrazole (117 g) were added sulfuric acid (91 g) and 10% palladium on carbon (58 g). The mixture was
25 hydrogenated under balloon pressure for 10 hours. The reaction mixture was filtered and the filtrate was concentrated in vacuo. To the concentrate was added isopropyl alcohol (2.3 L) and the mixture was stirred for 1 hour. The precipitated solid was collected by
30 filtration and dried to give 4,5-diamino-1-methylpyrazole sulfuric acid salt (158 g).

$^1\text{H-NMR}(\text{D}_2\text{O})$ δ 3.74 (3H, s), 7.80 (1H, s)

Preparation 44

- A solution of 4,5-diamino-1-methylpyrazole
35 sulfuric acid salt (158 g) in water (1.1 L) was neutralized to pH 6.9 with 4N aqueous sodium hydroxide solution, and dioxan (474 ml) was added to this solution. To the resulting mixture was added dropwise

phenyl chloroformate (124 g) maintaining pH of the mixture at 6.9 with 4N aqueous sodium hydroxide solution at a temperature below 10°C. The reaction mixture was stirred for 1 hour. The precipitated solid was

- 5 collected by filtration and dried to give 5-amino-1-methyl-4-phenoxy carbonylaminopyrazole (155 g).

$^1\text{H-NMR}$ (DMSO- d_6) δ 3.52 (3H, s), 5.00 (2H, brs), 7.10-7.50 (6H, m), 8.93 (1H, brs)

Preparation 45

- 10 To a suspension of 5-amino-1-methyl-4-phenoxy carbonylaminopyrazole (153.8 g) in tetrahydrofuran (1 L) were added triethylamine (67 g) and triphenylmethyl chloride (185 g) at room temperature. The mixture was stirred for 6.5 hours. To the reaction
15 mixture was added heptane (2.6 L) and the mixture was stirred for 1 hour. The precipitated solid was collected by filtration and washed with heptane-diisopropyl ether (1:1). The crude solid was suspended in water (3 L) and the suspension was stirred for 1 hour.
20 The solid was collected by filtration and dried to give 1-methyl-4-phenoxy carbonylamino-5-triphenylmethylaminopyrazole (253.6 g):

$^1\text{H-NMR}$ (DMSO- d_6) δ 2.74 (3H, s), 5.57 (1H, brs), 7.00-7.50 (21H, m), 8.12 (1H, brs)

25 Preparation 46

- To a suspension of 1-methyl-4-phenoxy carbonylamino-5-triphenylmethylaminopyrazole (253.6 g) in N,N-dimethylformamide (1.5 L) were added
triethylamine (59.5 g) and tert-butyl N-(2-aminoethyl) carbamate (94.2 g) in N,N-dimethylformamide
30 (254 ml). The mixture was stirred for 5 hours and poured into water (10.6 L). The slurry was stirred for 1 hour. The precipitated solid was collected by filtration and dried to give a crude product. The crude
35 product was suspended in N,N-dimethylformamide and the suspension was heated under reflux for 20 minutes. The suspension was cooled to ambient temperature over 4 hours. The solid was collected by filtration, washed

with acetonitrile and dried to give 4-[N-(2-tert-butoxycarbonylaminoethyl)carbamoylamino]-1-methyl-5-triphenylmethylaninopyrazole (261.2 g).

¹H-NMR(DMSO-d₆) δ 2.69 (3H, s), 2.90-3.05 (4H, m), 5.69 (1H, brs), 5.91-6.01 (1H, m), 6.74-6.81 (1H, m), 6.87 (1H, brs), 7.00 (1H, s), 7.10-7.30 (15H, m)

Preparation 47

To a solution of (Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetic acid (319 g, 642 mmol) in N,N-dimethylacetamide (1.5 L) were added potassium carbonate (113 g) and methanesulfonyl chloride (126 ml) under ice-cooling. The mixture was stirred at 10°C for 2 hours. The reaction mixture was added to a mixture of ethyl acetate and water. The organic layer was washed with water and brine to give an activated acid solution. On the other hand, a suspension of 4-methoxybenzyl 7β-amino-3-chloromethyl-3-cephem-4-carboxylate hydrochloride (300 g, 740 mmol) in a mixture of water (1 L) and ethyl acetate (1 L) was adjusted to pH 6 with triethylamine under ice-cooling. To the resulting mixture was dropwise added the above obtained activated acid solution at 10°C under stirring. Stirring was continued at 5-10°C for 1.5 hours keeping pH of the reaction mixture at 6 with triethylamine. The organic layer was separated, washed with water and brine, and evaporated in vacuo. The concentrate was poured into diisopropyl ether (15 L), and the resulting precipitate was collected by filtration and dried to give 4-methoxybenzyl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (495.7 g, yield 98.3%).

¹H-NMR(DMSO-d₆) δ 1.39 (9H, s), 1.44 (6H, s), 3.45-3.70 (2H, m), 3.76 (3H, s), 4.46 and 4.54 (1H, ABq, J=16Hz), 5.10-5.28 (2H+1H, m), 5.90 (1H, dd, J=4.9Hz, 8.5Hz), 6.94 (2H, d, J=8.7Hz), 7.36 (2H, d, J=8.7Hz), 8.18 (2H, brs), 9.52 (1H, d, J=8.5Hz)

Example 26

To a solution of 4-methoxybenzyl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate (150 g) in N,N-dimethylformamide (400 ml) was added 1,3-bis(trimethylsilyl)urea (225 g) and the mixture was stirred for 30 minutes. Potassium iodide (51.2 g) was added to this solution and the mixture was stirred for 30 minutes.

4-[N-(2-tert-Butoxycarbonylaminoethyl)-carbamoylamino]-1-methyl-5-triphenylmethylaminopyrazole (147 g) was dissolved in N,N-dimethylformamide (650 ml) at 78°C and the solution was cooled to 45°C. The solution was added to the solution of 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate obtained above. The reaction mixture was stirred at 35°C for 18.5 hours and poured into a mixture of ethyl acetate (2 L), water (1.8 L) and 20% aqueous sodium chloride solution (150 ml). The organic layer was washed with a mixture of 10% aqueous sodium thiosulfate solution (375 ml) and 20% aqueous sodium chloride solution (375 ml). The organic layer was washed successively with 10% aqueous sodium trifluoroacetate solution three times (750 ml x 3) and 20% aqueous sodium chloride solution (750 ml). The organic layer was concentrated in vacuo and precipitated 4-[N-(2-tert-butoxycarbonylaminoethyl)carbamoylamino]-1-methyl-5-triphenylmethylaminopyrazole was filtered off. The filtrate was further concentrated in vacuo to a volume of approximately 600 ml. This solution was added to diisopropyl ether and the suspension was stirred for 1 hour. The resulting solid was collected by filtration and dried. The solid was dissolved in dichloromethane (669 ml). To the solution were added anisole (223 ml) and trifluoroacetic acid (669 ml). The reaction mixture was stirred for 4 hours and poured into diisopropyl ether. The resulting solid was collected by filtration

and dried. This solid was suspended in water and pH of the suspension was adjusted to 7 with aqueous ammonia solution at a temperature below 10°C. The resulting precipitate was filtered off. The filtrate was
5 acidified to pH 1 with concentrated hydrochloric acid at a temperature below 10°C and the resulting precipitate was filtered off. The filtrate was chromatographed on Diaion® HP-20 (11 L) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 3.5 L in vacuo and
10 2M sulfuric acid (51 ml) was added. The mixture was lyophilized to give crude product (72.2 g).

The crude product (3 g) was purified by preparative high-performance liquid chromatography (HPLC) utilizing ODS column. The eluate containing a
15 desired product was concentrated in vacuo. The concentrate was adjusted to about pH 1 with concentrated hydrochloric acid and chromatographed on Diaion® HP-20 (400 ml) eluting with 20% aqueous 2-propanol. The eluate was concentrated to about 73 ml in vacuo and 2M
20 sulfuric acid (1.5 ml) was added. The mixture was further evaporated to a volume of approximately 12.5 ml and water (6 ml) was added. After addition of seed crystals (10 mg), which resulted in the precipitation of a white solid, the mixture was stirred at room
25 temperature for 1 hour. The mixture was further stirred at 5°C for 13 hours. 2-Propanol (20 ml) was added at 5°C over 20 minutes and the slurry was stirred at room temperature for 4 hours. 2-Propanol (20 ml) was added over 30 minutes and the slurry was stirred at room
30 temperature for 3 hours. The precipitated crystals were collected by filtration and dried to give 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-(3-amino-4-[N-(2-aminoethyl)carbamoylamino]-2-methyl-1-pyrazolio)methyl-
35 3-cephem-4-carboxylate hydrogen sulfate (1.51 g) as crystals.

¹H-NMR(D₂O) δ 1.61 (6H, s), 3.10-3.55 (6H, m), 3.71 (3H, s), 5.02 and 5.23 (2H, ABq, J=16.7Hz), 5.25 (1H, d,

J=4.9Hz), 5.87 (1H, d, J=4.9Hz), 7.91 (1H, s)

Preparation 48

A suspension of 4,5-diamino-1-methylpyrazole
sulfuric acid salt (20 g, 95.1 mmol) in triethylamine
5 (29.2 ml, 209 mmol) was stirred at 0°C for 10 minutes.
A mixture of acetic anhydride (9.87 ml, 105 mmol) and
formic acid (7.96 ml, 209 mmol) was stirred at 40°C for
30 minutes, cooled to 0°C, and added dropwise to the
above solution at 0°C. The whole mixture was stirred at
10 0°C for 2 hours. To the mixture was added brine, and
the whole mixture was extracted with tetrahydrofuran.
The organic layer was dried over magnesium sulfate and
evaporated under reduced pressure to give crude N-(5-
amino-1-methyl-1H-pyrazol-4-yl)formamide, which was used
15 in the next step without further purification.

Preparation 49

The crude product of N-(5-amino-1-methyl-1H-
pyrazol-4-yl)formamide (13.33 g, 95.1 mmol) was
dissolved in N,N-dimethylformamide (130 ml). To the
20 solution were added trityl chloride (29.2 g, 105 mmol),
triethylamine (66.3 ml, 476 mmol) and 4-
dimethylaminopyridine (465 mg, 3.8 mmol), and the
mixture was stirred at 60°C for 5 hours. To the
reaction mixture was added water, and the whole mixture
25 was extracted with ethyl acetate. The organic layer was
washed with water and brine, and dried over magnesium
sulfate. The solvent was evaporated under reduced
pressure to give a white solid. The solid was
trituated with ethyl acetate/diisopropyl ether (1:1) to
30 give N-[1-methyl-5-(tritylamino)-1H-pyrazol-4-
yl]formamide (19.18 g). The NMR spectrum of this
compound indicates the existence of its rotamer.
¹H-NMR(DMSO-d₆) δ 2.76 and 2.92 (3H, s), 5.56 and 5.84
(1H, s), 7.0-7.4 (16H, m), 7.66 (1H, d, J=1.7Hz), 8.3-
35 8.4 (1H, m)

ESI-MS: m/z=405.2 (M+Na)⁺

Preparation 50

To a solution of N-[1-methyl-5-(tritylamino)-1H-

pyrazol-4-yl]formamide (3.825 g, 10 mmol) in N,N-dimethylformamide (66 ml) was added sodium hydride (264 mg, 60% oil suspension, 11 mmol) under a nitrogen atmosphere at 0°C under stirring. The mixture was stirred at 0°C for 15 minutes. To the mixture were added tert-butyl N-(3-bromopropyl)carbamate (2.62 g, 11 mmol) in N,N-dimethylformamide (10 ml) and sodium iodide (1.65 g, 11 mmol). The mixture was warmed to room temperature and stirred for 2 hours. 10% Aqueous potassium hydrogen sulfate solution (5 ml) was added, and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel eluting with methylene chloride/ethyl acetate (4:1) to give tert-butyl 3-(formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino)propylcarbamate (2.714 g, yield 50.3%). The NMR spectrum of this compound indicates the existence of its rotamer.

¹H-NMR(DMSO-d₆) δ 1.37 and 1.39 (9H, s), 2.6-2.9 (6H, m), 2.89 (3H, s), 5.34 and 6.01 (1H, s), 6.6-6.9 (1H, m), 7.0-7.4 (15H, m), 7.5-7.6 (1H, m), 7.95 (1H, s)

ESI-MS: m/z=562.3(M+Na)⁺

Example 27

7β-[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(3-aminopropyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

The title compound was obtained from benzhydryl 7β-[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-iodomethyl-3-cephem-4-carboxylate and tert-butyl 3-(formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino)propylcarbamate in the same manner as in Example 1. The NMR spectrum of this compound indicates the existence of its rotamer.

¹H-NMR(D₂O) δ 1.53 (6H, s), 1.7-2.1 (2H, m), 2.9-3.9 (9H,

m), 4.97 and 5.20 (2H, ABq, J=15.2Hz), 5.26 (1H, d, J=4.8Hz), 5.84 (1H, d, J=4.8Hz), 8.0-8.3 (2H, m)

Example 28

To a suspension of 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)-acetamido]-3-{3-amino-4-[(3-aminopropyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (140 mg, 0.21 mmol) in methanol (2.6 ml) was added concentrated hydrochloric acid (0.176 ml, 2.1 mmol) at room temperature, and the mixture was stirred for 6.5 hours. To the reaction mixture was added sodium hydrogen carbonate (177 mg, 2.1 mmol), and the mixture was purified by preparative HPLC (ODS column, acetonitrile/phosphate buffer (pH 7)=5:95). The eluate containing a desired product was evaporated to remove acetonitrile, acidified with diluted hydrochloric acid and chromatographed on Diaion® HP-20 eluting with 20% aqueous 2-propanol. The eluate was concentrated under reduced pressure and freeze-dried to give 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(3-aminopropyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate (39 mg).

¹H-NMR(D₂O) δ 1.52-1.54 (6H, m), 1.95 (2H, tt, J=7.3Hz, 7.3Hz), 3.0-3.2 (4H, m), 3.16 and 3.38 (2H, ABq, J=17.7Hz), 3.68 (3H, s), 4.89 and 5.11 (2H, ABq, J=15.6Hz), 5.22 (1H, d, J=4.8Hz), 5.83 (1H, d, J=4.8Hz), 7.59 (1H, s)

ESI-MS: m/z=636.3(M-H)⁻

Preparation 51

tert-Butyl 2-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}ethylcarbamate

The title compound was obtained from N-[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]formamide and tert-butyl N-(2-bromoethyl)carbamate in the same manner as in Preparation 50.

IR(KBr) 1709, 1670, 1170, 704 cm⁻¹

¹H-NMR(DMSO-d₆) δ 1.35 and 1.36 (9H, s), 2.65 and 2.75

(3H, s), 2.73-2.90 (4H, m), 5.45 and 6.02 (1H, s), 6.78 and 6.88 (1H, t-like), 7.05-7.30 (15H, m), 7.31 and 7.57 (1H, s)

ESI-MS: $m/z=426.3 (M+H^+)$, $548.3 (M+Na^+)$

5 Example 29

7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

10 The title compound was obtained from benzhydryl 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-tert-butoxycarbonyl-1-methylethoxyimino)acetamido]-3-chloromethyl-3-cephem-4-carboxylate and tert-butyl 2-{formyl[1-methyl-5-(tritylamino)-1H-pyrazol-4-yl]amino}ethylcarbamate in the same manner as in Example 1.

IR(KBr) 1770, 1675, 1653, 1597 cm^{-1}

1H -NMR(DMSO- d_6) δ 1.53 (6H, s), 3.12-3.78 (4H, m), 3.77 and 3.78 (3H, s), 3.86-3.96 (2H, m), 5.00 and 5.19 (2H, ABq, $J=15.2Hz$), 5.28 (1H, d, $J=4.8Hz$), 5.86 (1H, d, $J=4.8Hz$), 8.15 and 8.18 (1H, s), 8.19 and 8.33 (1H, s)

ESI-MS: $m/z=652.2 (M+H^+)$

Example 30

25 7 β -[(Z)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate

30 The title compound was obtained from 7 β -[(Z)-2-(5-amino-1,2,4-thiadiazol-3-yl)-2-(1-carboxy-1-methylethoxyimino)acetamido]-3-{3-amino-4-[(2-aminoethyl)(formyl)amino]-2-methyl-1-pyrazolio}methyl-3-cephem-4-carboxylate in the same manner as in Example 28.

IR(KBr) 1770, 1651, 1593 cm^{-1}

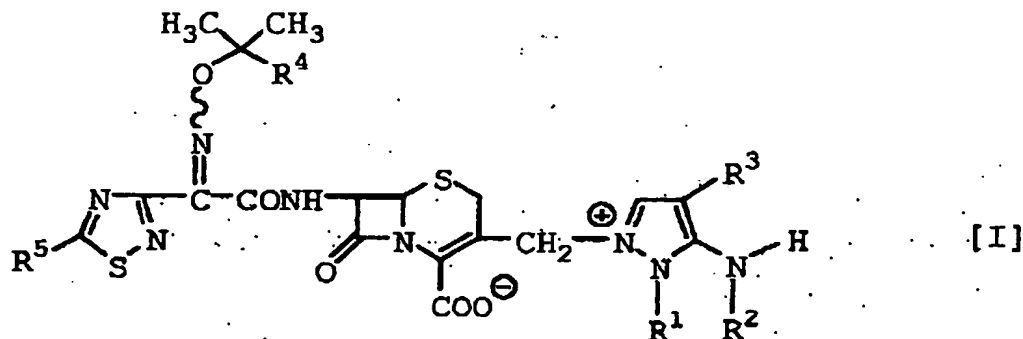
35 1H -NMR(DMSO- d_6) δ 1.53 (3H, s), 1.59 (3H, s), 3.13-3.26 (4H, m), 3.26 and 3.39 (2H, ABq, $J=17.8Hz$), 3.68 (3H, s), 4.87 and 5.11 (2H, ABq, $J=15.7Hz$), 5.25 (1H, d, $J=4.8Hz$), 5.84 (1H, d, $J=4.8Hz$), 7.63 (1H, s)

ESI-MS: $m/z=622.2 (M-H^-)$

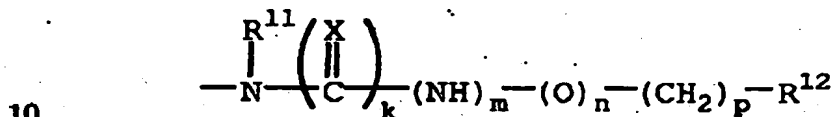
- 71A -

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or
5 step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

1. A compound of the formula [I]:



R¹ is lower alkyl or hydroxy(lower)alkyl, and
R² is hydrogen or amino protecting group, or
R¹ and R² are bonded together and form lower alkylene;
R³ is



15 p is 0, 1, 2 or 3;

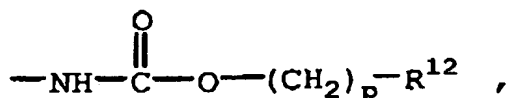
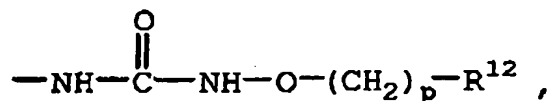
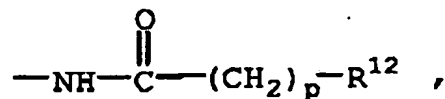
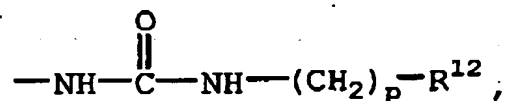
or a pharmaceutically acceptable salt thereof.

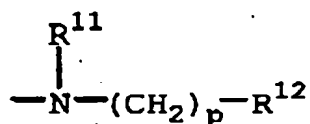
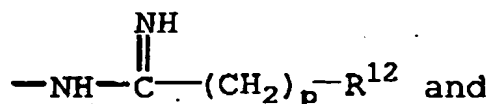
or a pharmaceutically acceptable salt thereof.

3. The compound of claim 2 wherein
 R^1 is lower alkyl or hydroxy(lower)alkyl, and
 R^2 is hydrogen, aryl(lower)alkyl, lower alkanoyl or
lower alkoxycarbonyl, or
5 R^1 and R^2 are bonded together and form lower alkylene;
 R^4 is carboxy or lower alkoxycarbonyl;
 R^5 is amino, lower alkanoylamino or lower
alkoxycarbonylamino;
 R^{11} is hydrogen, lower alkanoyl or lower alkoxycarbonyl;
10 and
 R^{12} is amino, lower alkanoylamino or lower
alkoxycarbonylamino,
or a pharmaceutically acceptable salt thereof.

15 4. The compound of claim 3 wherein
 R^1 is lower alkyl or hydroxy(lower)alkyl, and
 R^2 is hydrogen, or
 R^1 and R^2 are bonded together and form lower alkylene;
 R^4 is carboxy;
20 R^5 is amino;
 R^{11} is hydrogen or lower alkanoyl; and
 R^{12} is amino,
or a pharmaceutically acceptable salt thereof.

25 5. The compound of claim 1 wherein
 R^3 is selected from the group consisting of



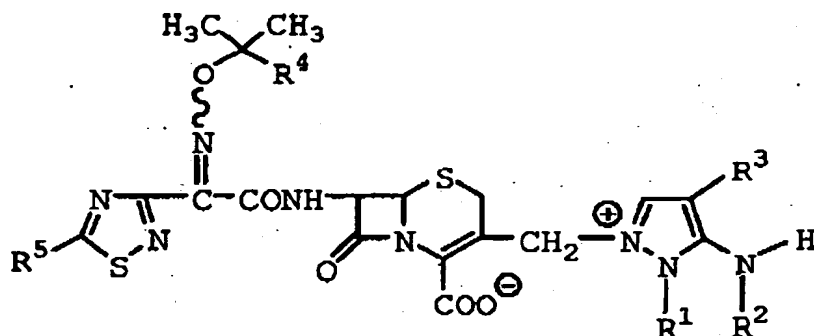


wherein R^{11} , R^{12} and p are each as defined in claim 1,
or a pharmaceutically acceptable salt thereof.

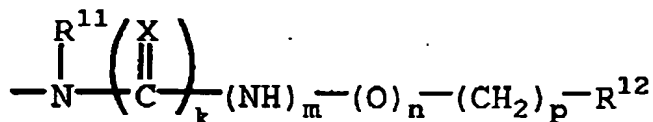
- 5 6. The compound of claim 5 wherein
 R^{11} is hydrogen, lower alkanoyl or lower alkoxy carbonyl;
 and
 R^{12} is amino, lower alkanoylamino or lower
 alkoxy carbonylamino,
 10 or a pharmaceutically acceptable salt thereof.

7. The compound of claim 6 wherein
 R^{11} is hydrogen or lower alkanoyl; and
 R^{12} is amino,
 15 or a pharmaceutically acceptable salt thereof.

8. A process for preparing a compound of the formula
 [I]:



- 20 wherein
 R^1 is lower alkyl or hydroxy(lower)alkyl, and
 R^2 is hydrogen or amino protecting group, or
 R^1 and R^2 are bonded together and form lower alkylene;
 R^3 is



wherein X is O or NH,

R¹¹ is hydrogen or amino protecting group,

R¹² is amino or protected amino,

5 k, m and n are independently 0 or 1, and

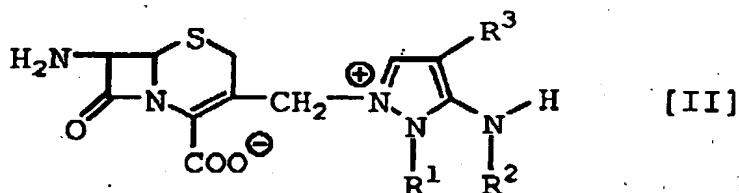
p is 0, 1, 2 or 3;

R⁴ is carboxy or protected carboxy; and

R⁵ is amino or protected amino,

or a salt thereof, which comprises

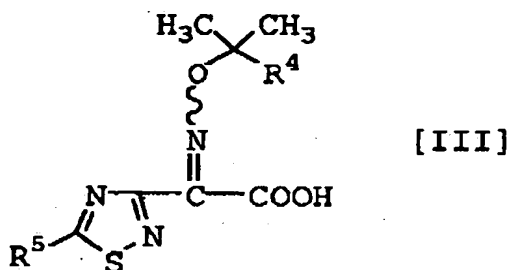
10 (1) reacting a compound of the formula [II]:



wherein R¹, R² and R³ are each as defined above, or its

15 reactive derivative at the amino group, or a salt

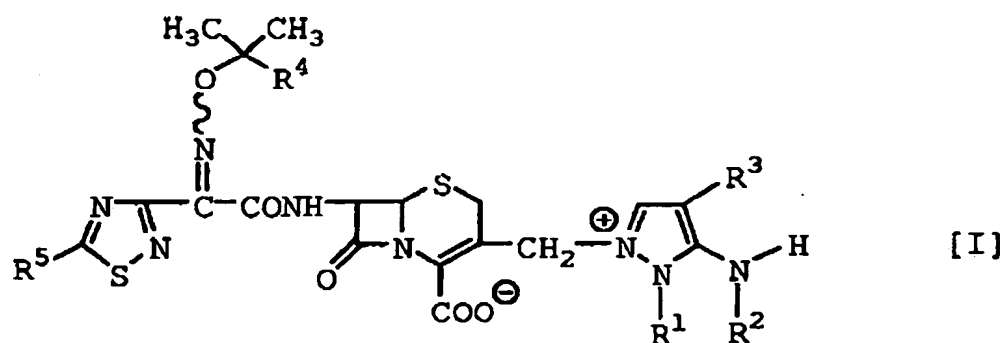
thereof with a compound of the formula [III]:



wherein R⁴ and R⁵ are each as defined above, or its

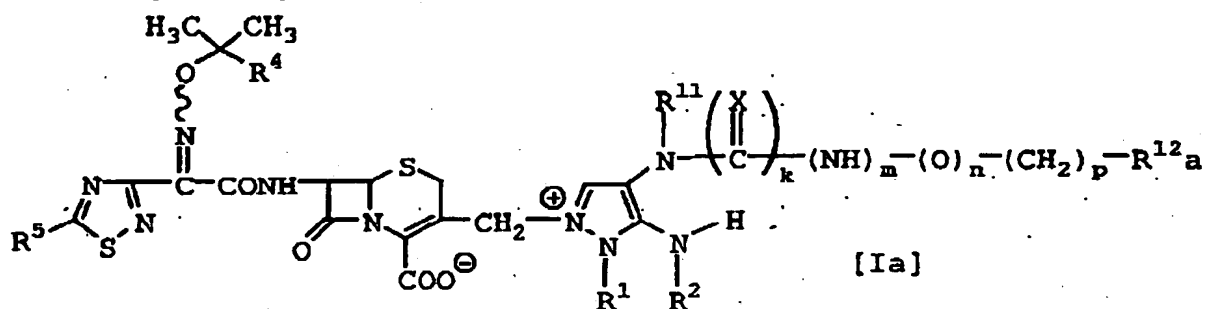
reactive derivative at the carboxy group, or a salt

20 thereof to give a compound of the formula [I]:



wherein R^1 , R^2 , R^3 , R^4 and R^5 are each as defined above, or a salt thereof, or

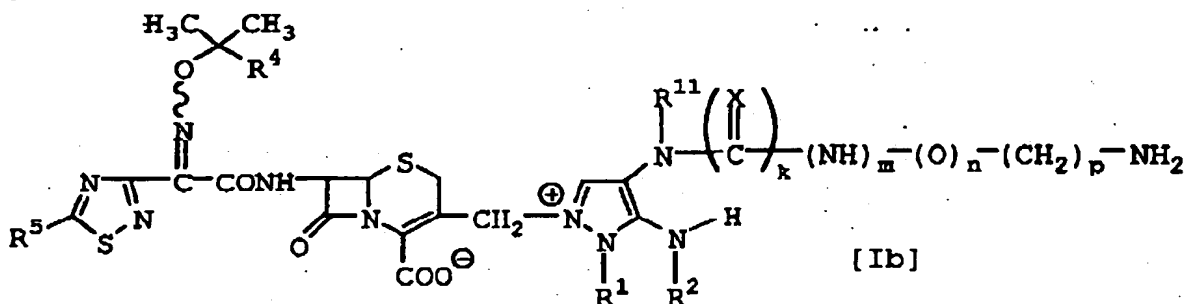
(2) subjecting a compound of the formula [Ia]:



5

wherein R^1 , R^2 , R^4 , R^5 , R^{11} , X , k , m , n and p are each as defined above, and R^{12a} is protected amino, or a salt thereof to elimination reaction of the amino protecting group to give a compound of the formula [Ib]:

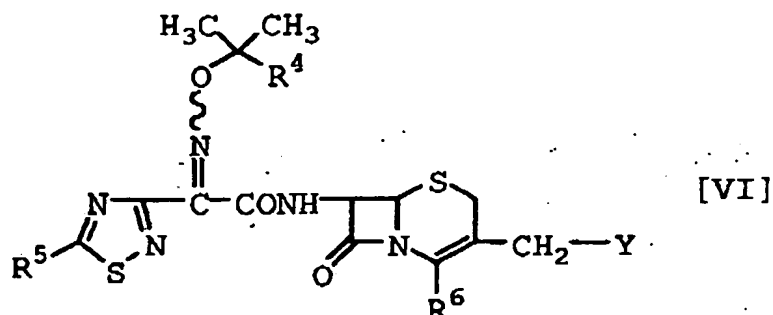
10



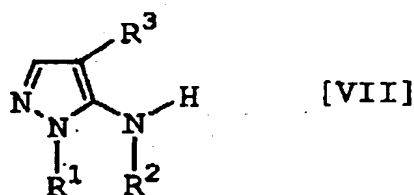
wherein R^1 , R^2 , R^4 , R^5 , R^{11} , X , k , m , n and p are each as defined above, or a salt thereof, or

(3) reacting a compound of the formula [VI]:

15

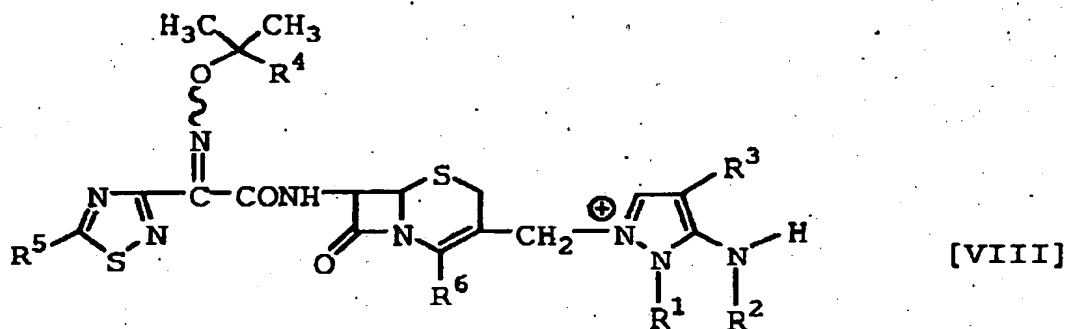


wherein R⁴ and R⁵ are each as defined above, R⁶ is protected carboxy, and Y is a leaving group, or a salt thereof with a compound of the formula [VII]:

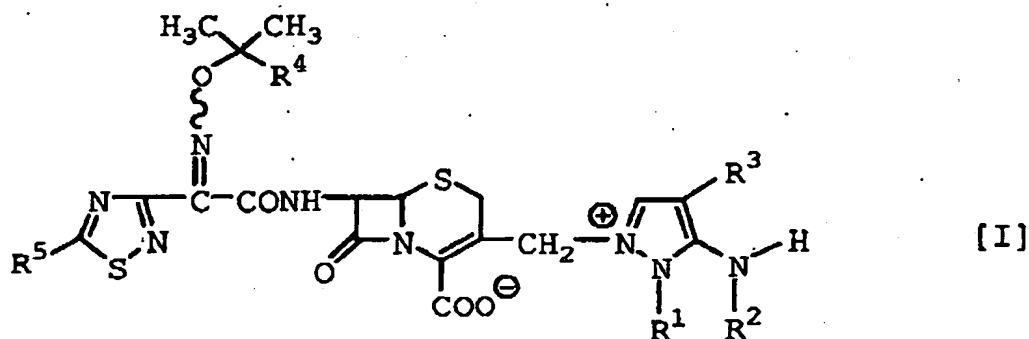


5

wherein R¹, R² and R³ are each as defined above, or a salt thereof to give a compound of the formula [VIII]:



10 wherein R¹, R², R³, R⁴, R⁵ and R⁶ are each as defined above, or a salt thereof, and
subjecting the compound of the formula [VIII] or a salt thereof to elimination reaction of the carboxy protecting group, to give a compound of the formula [I]:



wherein R¹, R², R³, R⁴ and R⁵ are each as defined above,
or a salt thereof.

5 9. A pharmaceutical composition comprising a compound
of claim 1 or a pharmaceutically acceptable salt thereof
in admixture with a pharmaceutically acceptable carrier.

10 10. A compound of claim 1 or a pharmaceutically
acceptable salt thereof for use as a medicament.

11. A compound of claim 1 or a pharmaceutically
acceptable salt thereof for use as an antimicrobial
agent.

15 12. Use of a compound of claim 1 or a pharmaceutically
acceptable salt thereof for manufacture of a medicament
for treating infectious diseases.

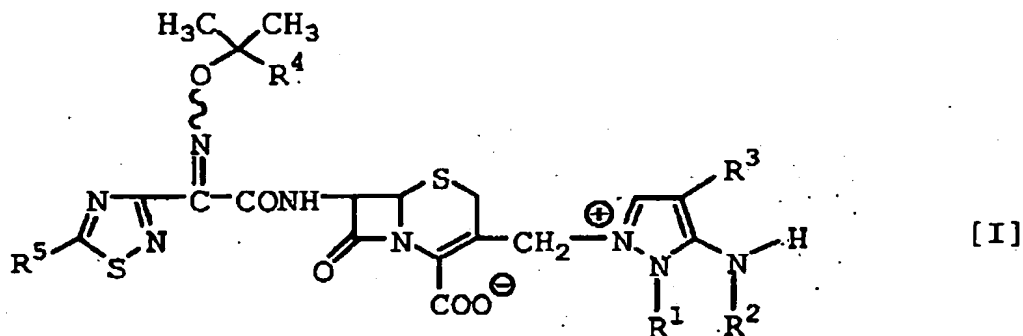
DATED this 30th day of October, 2002

Fujisawa Pharmaceutical Co., Ltd. and
Wakunaga Pharmaceutical Co., Ltd

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Patent Attorneys for the Applicant

ABSTRACT

The present invention relates to a compound of the formula [I]:



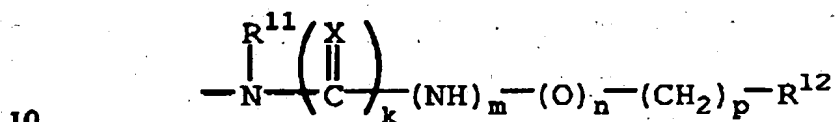
5 wherein

R¹ is lower alkyl or hydroxy(lower)alkyl, and

R² is hydrogen or amino protecting group, or

R¹ and R² are bonded together and form lower alkylene;

R³ is



wherein X is O or NH,

R¹¹ is hydrogen or amino protecting group,

R¹² is amino or protected amino,

k, m and n are independently 0 or 1, and

15 p is 0, 1, 2 or 3;

R⁴ is carboxy or protected carboxy; and

R⁵ is amino or protected amino,

or a pharmaceutically acceptable salt thereof, a process

for preparing a compound of the formula [I], and a

20 pharmaceutical composition comprising a compound of the formula [I] in admixture with a pharmaceutically acceptable carrier.